General Introduction

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Biomarker research in mental disorders
Linking biomarkers to etiology

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Manuscript in preparation

Abstract

Amongst complex diseases, psychiatric disorders are probably the least understood. Unfortunately, at the same time they contribute enormously to psychological, social and economic suffering on a global and individual level. Especially in third-world countries an affected relative is really detrimental for a household. It is remarkable that despite the wide availability of psychiatric services and psychiatrists in Western countries, under-diagnosis and under-treatment are still common. This may be related to currently available diagnostic and treatment modalities that are far from perfect. A largely unknown disease etiology and a diagnostic system that is based on the observation of symptom clusters can be held, at least partially, responsible for this situation. In view of the large number of epidemiological studies, studies of animal models for anxiety, depression and addiction and extensive research into mental disorders, one might expect otherwise. Today most new medication for psychiatric disorders is still being discovered by serendipity in spite of our growing knowledge of molecular and cellular processes involved in neural signaling and neuronal development. In most cases there is no effective medication without harmful side-effects that can cure neurodevelopmental and mental disorders.

Research has, however, enriched our knowledge with the identification of psychosocial and cultural risk factors and early-life events that increase the risk of developing a mental disorder. Genetic analysis has yielded some candidate genes that, depending on the disorder in question, have a rather small to negligible contribution to the overall risk. Notwithstanding all these efforts no single gene has thus far been identified that can explain the complex etiology of a mental disorder except for Rett's syndrome. Altered expression of genes caused by epigenetic deregulation rather than mutations of the DNA sequence may be more relevant for the development of mental disorders. Upcoming unbiased, non-hypothesis driven approaches based on genomics, transcriptomics, proteomics and metabolomics technologies hold promise to increase our comprehension of mental disorders through the (expression-) profiling of hundreds to thousands of genes, gene-transcripts (mRNA), proteins and metabolites, respectively. For the interpretation of the results, powerful bioinformatics approaches are crucial. Integration of these '-omics' results in comprehensive functional correlation networks offer the possibility to study psychiatric diseases in a systems biology approach. By doing so it is expected that new prognostic, diagnostic and therapeutic biological markers or panels of markers will be discovered, because 'childhood-diseases' that have impaired biomarker research in cancer seem to have been dealt with.

New markers or marker panels should have superior sensitivity and specificity and should preferably relate in a causative manner to the organ, tissue, cell or

molecular pathway that is involved in the pathophysiology of the given disease. This may also simplify the present picture of mental disorders that is defined by phenomenological observations into biochemically related classes. In the end it may even be possible to make mental disorders run a less severe course, to prevent or delay the onset, to decrease the impact of environmental risk-factors, to identify highly-susceptible individuals, and to prevent or even cure mental disorders in generations to come.

1. Introduction

The world health organization (WHO) reported in 2001 that 450 million (10%) of all people suffer from mental or neurological disorders or from psychosocial problems such as those related to alcohol and drug abuse [1]. One in 4 people will be affected by a neuropsychiatric disorder at some stage of their life [1]. Men and women are equally affected with some exceptions, such as a higher prevalence of alcohol and substance abuse disorders in men and of unipolar depressive disorder in women [1]. Other examples of neuropsychiatric disorders include bipolar affective disorder (BD), schizophrenia, epilepsy, Alzheimer's and other dementias, post traumatic stress disorder (PTSD), obsessive and compulsive disorder, panic disorder, and primary insomnia. All of these involve cognitive, emotional, behavioral and interpersonal impairments. It is alarming that the largest portion of individuals with neuropsychiatric disorders remains untreated [2]. Next to the enormous suffering of families with affected relatives there is the associated economic burden of mental disorders caused by health care and social service needs, lost employment and reduced productivity (e.g. mental health problems account for 35–45% of absenteeism from work [3]), impact on families and caregivers, levels of crime and public safety, and the negative effect of premature mortality.

The high life-time prevalence puts also a significant burden on primary care, because 24% of all patients attend these facilities because of a mental disorder [4]. Mental disorders thus contribute largely to the global burden of disease and health care costs. Importantly, projections based on figures of the 1990 WHO Global Burden of Disease Study in 1997 by Murray and Lopez [5] note that there will be a 40% increase (from 10.5 to 14.7%) of disability-adjusted life years (DALY; see 'glossary' at the end of the 'References' section) caused by neuropsychiatric disorders from 1990 to 2020. An update of these projections, based on figures of the WHO from 2002, even shows unipolar depressive disorders will be ranked as the second leading cause (5.7%) of DALYs in 2030 [6].

In regions with high-income countries such as Europe, neuropsychiatric disorders account for over 40% of chronic diseases and these disorders are the greatest cause of years lived with disability (YLD). With 19.5% of DALYs, they come in second after cardiovascular disease [3]. In westernized countries unipolar depressive disorder will become the leading cause of illness, while in low-income countries it will rank third [6] on the list of DALY causatives.

Few reports are available regarding the economic impact of mental disorders in developing countries. However, reports from industrialized countries show, for example, that in the U.S.A. 7% of total health care expenditures are spent on mental illness and that the total cost of mental disorders is about 2% (38.4 billion €)

of the U.S. gross domestic product [7]. Europe's mental health care budgets constitute on average 5.8% of total health expenditure with a wide variation between countries (0.1% to 12%) [3]. The cost of mental disorders constitutes a significant proportion of the overall economy and the negative economic consequences of mental illness greatly exceed the cost of treatment. It is thus important to prevent and treat mental illness for these two reasons.

One of the ten recommendations in the world health report of 2001 by the WHO is to support research into biological and psychosocial aspects of mental health in order to increase the understanding of mental disorders and to develop more effective interventions [1]. The European Union adds to this that interventions should also be evaluated for their cost–effectiveness [3]. A recent cost-effectiveness study [8] shows that current treatments, such as first-generation antipsychotic, antidepressant and antianxiolytic drugs combined with psychosocial treatment, are very cost-effective and steps should be undertaken to increase treatment coverage and adherence. However, even if unlimited funding was available and all individuals affected by a psychiatric disease were treated optimally, about 60% of the burden would remain unavertable [9]. The burden of mental illness is especially heavy in developing countries in which poverty, HIV/AIDS, violence, prejudice etc. use up most of the resources. Treating mental illness is often seen as a luxury in these countries [10]. These problems need to be addressed before findings of more applied and fundamental research can be implemented.

Although research focusing at biological aspects of mental health may not find its way into pharmacological interventions directly, it will probably contribute to the understanding of the cause, course and outcome of disease. The use of biomarkers, i.e. molecules that indicate a physiological alteration due to development of disease for preventive, diagnostic, prognostic and therapeutic purposes, is relatively new, but this field of research has gained much interest in the past decades. This article aims to review the role and potential of biomarkers and biomarker research in psychiatric diseases, with the emphasis on schizophrenia and pervasive developmental disorders (PDD; see 'glossary') /autism spectrum disorder (ASD; see 'glossary'). The latter two terms will be interchangeably used throughout this review. Although this review will cover many topics regarding the epidemiology and etiology of mental disorders, it will not be exhaustive in this respect. Many comprehensive and recommendable reviews regarding specific topics discussed in this review have been published and the authors will refer the reader to these reviews for more detailed overviews where appropriate.

2. Epidemiology and economic costs

A more scientific view on the causes and symptoms of psychiatric diseases was adapted after superstition, e.g. demonic possession and witchcraft, had been abandoned. The puritan clergyman Cotton Mather (1663-1728) was one of the first to advance physical explanations for mental illnesses in Renaissance Europe [11], while the Arab physician Rhazes already described definitions, symptoms, and treatments for mental illness in the 10th century [12]. Acceptance that mental illness is caused by an unlucky combination of genetic background and environmental factors made room for epidemiological studies that identified gradients across time and/or space. From these studies underlying risk factors were identified. MEDLINE has cited studies on the epidemiology of depression, bipolar disorder, schizophrenia and developmental disorders since the 1970s.

Proper estimation of incidence and prevalence rates, and detection of trends, have in the past been hampered by different ascertainment strategies across epidemiological studies. With the global implementation and acceptance of classification systems for mental disorders (i.e. Text-Revision of the 4th (IV) edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) of the American Psychiatric Association [13], and the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) of the WHO [14]) more reliable numbers on prevalence and incidence have become available. The bias of an underestimation of the prevalence of psychiatric diseases in the developing world may nevertheless be present, because many epidemiological studies reported in languages other than English, have not been accounted for in systematic reviews in the past. Up to a decade ago, it was, for example, believed that schizophrenia was randomly distributed across different cultures and regions. However, recent epidemiological data show otherwise [15].

The many evolution-based theories that are founded on the dogmatic belief that the incidence of schizophrenia is invariant across time and place, are currently being challenged. Systematic reviews on incidence and prevalence estimates of schizophrenia show a median (10-90th percentile) incidence of 15.2 (7.7-43.0) per 100,000 persons [16], and a median (10-90th percentile) lifetime prevalence of 4.0 ‰ (1.6-12.1) [17]. It seems that about 7-8 individuals per 1,000 will develop schizophrenia during their lifetime, which is referred to as the lifetime morbid risk (LMR; see 'glossary'). In addition, the incidence estimates showed that males have a 1.4-times higher risk to develop schizophrenia than females and that migrants have a 4.6-times higher risk than native-born individuals. Living in an urban area also increases the risk of developing schizophrenia compared to living in mixed urban/rural sites [16]. Remarkably, there were no differences in prevalence estimates for different gender and urbanicity status [17]. The investigators observed

a 1.8-times higher prevalence in migrants compared to native-born individuals [17]. Developing nations had a lower prevalence of schizophrenia than developed nations, but this finding should be interpreted with caution, because a country's status of development was only based on a single crude economic variable (per capita gross national product). More research into factors that differentially influence the course of illness in men and women around the world, especially from poorer countries, is therefore warranted [17]. Risk factors associated with the incidence of schizophrenia were not fully congruent with those for prevalence. More importantly, these results call for the unraveling of factors that cause a differential course of schizophrenia between risk groups. For example, the fact that high latitude is associated with a higher prevalence of schizophrenia [18] is intriguing.

The economic burden of schizophrenia in Europe [19] and in the U.S. [20] (direct medical, non-medical and indirect costs) associated with psychotic disorders are respectively estimated to 35 billion € and 32.5 billion $ per year, which is primarily due to the early onset in adulthood and the fact that 2/3 of affected individuals have persistent and/or fluctuating symptoms despite optimal treatment [13]. The cost per capita spent on care for patients with schizophrenia are estimated at 1.1 million $ in the U.S. and 2.3 million $ in Canada [21]. Cost-effectiveness studies are necessary to maintain mental health care accessible for large groups of patients.

Despite its lower prevalence compared to psychotic disorders, pervasive developmental disorders (PDD or ASD) are also characterized by a significant burden of disease and economic cost, because of their early onset, lost productivity and required adult care. PDDs have a prevalence rate of about 60 persons per 10,000 [22] and the prevalence of autistic disorder and Asperger's disorder are estimated at about 13/10,000 and 3/10,000 [22], respectively. Childhood disintegrative disorder (CDD) is rarer with a prevalence of about 0.2/10,000 [22]. Rett's disorder seems to have a genetic origin, i.e. mutations of the methyl-CpG-binding protein 2 (MECP2) gene cause the majority of the cases of Rett syndrome (80% of affected females [23]) via an influence on chromatin remodeling [24]. A more recent systematic review of PDD prevalence studies showed slightly lower prevalence estimates of 7.1/10,000 for autism and 20/10,000 for ASD [25]. The 60% of variation in prevalence estimates between studies was attributed to changing diagnostic criteria, age of the sample, urban or rural location of sampling and retrospective or prospective case-assessment [25]. The Center for Disease Control in the U.S. [26] found that 5.2-7.6 of 1,000 children aged 8 years have ASD. This CDC study group also noted a trend for non-Hispanic white children to have slightly higher prevalence estimates than non-Hispanic black children. It is unclear why

prevalence estimates varied by race/ethnicity. Overall, their data also corroborate the notion that more males than females are affected (male-female ratio: 3.4-4.2 / 1) [26]. Interestingly, females (58%) were more prone to be cognitively impaired than males (42%) [26]. There is an apparent increase in incidence of PDD [26;27], but it is disputed whether this trend can be accounted for by other than methodological factors and increased awareness [22;27]. However, environmental risk factors cannot be ruled out. It is important for decision makers in health care to acknowledge this increasing trend. The CDC urges policy makers to improve early identification of ASD. In the future results from developmental epidemiology can contribute to the understanding of psychopathology as well as to the identification of environmental risk factors. Research into gene-environment correlations and interactions give hope that we can reduce the burden of child and adolescent mental illness by devising preventive and therapeutic measures [28].

Up-to-date information about prevalence rates is important because the societal economic costs, including education and treatments for children with ASD, amount to approximately 35 billion $ per year [29]. Total expenditures per 10 000 covered lives associated with ASD increased 142.1% over a 5-year period [30], because of a 20% increase in average health care expenditures from 2000 to 2004 and rising prevalence rates. Ironically, ASD creates a smaller burden on health insurances than other childhood disorders such as mental retardation, because of the relatively lower prevalence.

The lifetime per capita incremental societal cost of autism is $3.2 million [31]. Earlier identification and more proactive treatment will increase the burden of autism on the health care system, so efforts should be made to ensure that access to care for this vulnerable population is not compromised. Overall the utilization and cost of health care are significantly higher for children with PDD compared with children without PDD, underscoring the need to find more appropriate treatment options including biomedical approaches that target the core PDD symptoms [32]. Biomarker research into the causes, traits and treatment options of children with PDD should be stimulated by governmental institutions at least for matters of cost-effectiveness.

3. Diagnosis and classification

The statement that “current (diagnostic) criteria reflect perceived similarity of symptoms and prognoses, which is potentially influenced not only by actual etiological similarity, but also by the cultural and inherent person-perception biases of those perceiving the sufferer, and the categorization demands of legal, medical and research systems” [33] is both frustrating as well as liberating, because it might explain why many findings are only relevant for subgroups of patients with a

certain psychiatric diagnosis. It also stresses the need for other than categorical ways to characterize the different symptom dimensions of mental disorder, e.g. by endophenotypes [34]. Endophenotypes (see 'glossary') may provide an alternative approach to observation-based classification systems of heterogeneous disorders. An example of an endophenotype is the inability of schizophrenia patients and their unaffected family members to avoid looking at a visual cue that they have been told to ignore (antisaccade eye movement task). By definition only, endophenotypes differ from biological markers by the fact that the latter lack a (known) genetic basis. However, the question is to what extent and for what means the difference between endophenotypes and biological markers should be upheld.

3.1. Schizophrenia

Schizophrenia [35] is a disabling mental disorder that is caused by disruptions in thought processes. It is characterized by psychosis, apathy, social withdrawal, and cognitive impairment [13]. The positive symptoms of schizophrenia, including false beliefs (delusions) and perceptual experiences not shared by others (hallucinations), and bizarre behavior, are most prominent during a psychotic episode, whereas its negative symptoms, including blunted affect, apathy (loss of interest and motivation), anhedonia (inability to experience pleasure from normal activities) and alogia (diminished speech content), are more persistent [36]. These negative symptoms and deficits in cognition, including problems in attention and concentration, psychomotor speed, learning and memory, and executive functions are strongly associated with impaired psychosocial functioning [37;38], which is present in the prodromal phase of a psychosis [39]. The result is that most patients with schizophrenia have impaired functioning at work or school, in parenting, personal care, independent living, interpersonal relationships and leisure time even before their first psychotic episode [39]. Definitive diagnosis is usually assigned during hospital admission for a psychosis and a follow-up of at least 6 months is generally required [13].

Assignment of schizophrenia is done using either the DSM-IV-TR [13] or ICD-10 [14] criteria. These classification systems objectively define symptoms and characteristic impairments of schizophrenia in a similar way and the reliability of diagnosis between the two systems is high [40], even though a narrower definition of the disorder is used in the DSM-IV-TR. Temporal diagnostic consistency seems moderate (70%) with the highest variability immediately after onset of the disorder in outpatient and emergency settings [41]. However, this is far better than for most mental disorders, e.g. the temporal consistency of specific personality disorder is estimated at only 30% [41]. There is growing support for the view that these disorders should not be seen as discrete 'disease entities' but rather as dimensions of

continuous variations [42]. While the onset of schizophrenia occurs in the 2nd or 3rd decade of life, subtle abnormalities of cognition, social interaction, motor function and physical morphology are frequently observed in individuals who later develop schizophrenia [43], which is suggestive of a developmental vulnerability. The question arises whether there is a gold standard for the assignment of a mental disorder and so whether current treatment recommendations and interventions are always appropriate. The holy grail of biological psychiatry, a lab test for mental disorders [44], remains difficult to attain, because it relies on widely used classification systems of which the short-term consistency seems poor to at most moderate [41]. Multidisciplinary biomarker research may hold the promise of improving classification and follow-up of therapeutic efficacy.

3.2. Autism

Autism and the other PDDs were first described in 1943 by Kanner [45]. Since then, its criteria and ways of assignment have changed considerably. The term ASD is often used to refer to autistic disorder, PDD-NOS and Asperger's disorder, while the term PDD additionally comprises Rett's disorder and CDD [13]. Pervasive developmental disorders are characterized by severe and pervasive qualitative impairment in several areas of development: reciprocal social interaction skills, communication skills, or the presence of a restricted, stereotyped, repetitive repertoire of behavior, interests and activities [13;14]. Children with PDD also suffer frequently from mental retardation (30-70%), seizures (25%), hyperactivity and other behavioral problems [46].

A failure to develop joint attention, including a child's ability to share interests, pleasurable experiences or requests by using gestures or verbal communication in combination with eye contact, is one of the earliest indicators of autism [47]. Impairments are usually evident from about 18 months of age [48]. Early identification of children with PDD is important because early intervention is more effective in children with autism than in children with other developmental disabilities [49] and it helps minimizing the impact on the family. Having a PDD leads to major difficulties in daily living, school and work performance and most families are confronted with extraordinary demands on their time, energy and financial resources.

In addition to general medical evaluation, children are evaluated for the presence/absence of a PDD (categorical diagnosis), different dimensions of PDD (e.g. intellectual functioning, language, behavior) and the individual's most disruptive symptoms, which then become the focus of treatment [46]. Assignment of diagnosis usually involves a 2-level approach, with additional, more specific screening for autism of children that fail routine developmental screening [46;48].

Information from parents, teachers and pediatric clinicians working in primary care settings about the developmental profile in conjunction with standardized instruments is used to assign the clinical diagnosis, since DSM-IV TR criteria, which are considered the gold standard for ASD diagnosis, leave too much latitude for clinical judgment [48]. Autism-specific diagnostic tools are the Autism Diagnostic Interview (ADI-R, [50]) and the Autism Diagnostic Observation Schedule (ADOS, [51]). The ADI-R is a standardized investigator-based semi-structured interview that is applicable from about 18 months into adulthood. It aims to provide data on the behavior of a child or young adult to differentiate between autism and other developmental disorders [50]. It is, however, not suitable for children with a mental age below 2 years [52]. The ADOS is a semi-structured observational instrument based on DSM-IV criteria, which is used to differentiate between autism, PDD-NOS and other developmental disorders by assessment of social interaction, communication, play and imaginative use of materials [51]. The ADOS is highly sensitive in classifying children with ASD in their 2nd and 3rd year of life but its specificity is poor [52]. Revision of ADOS algorithms is ongoing to improve the diagnostic validity [53].

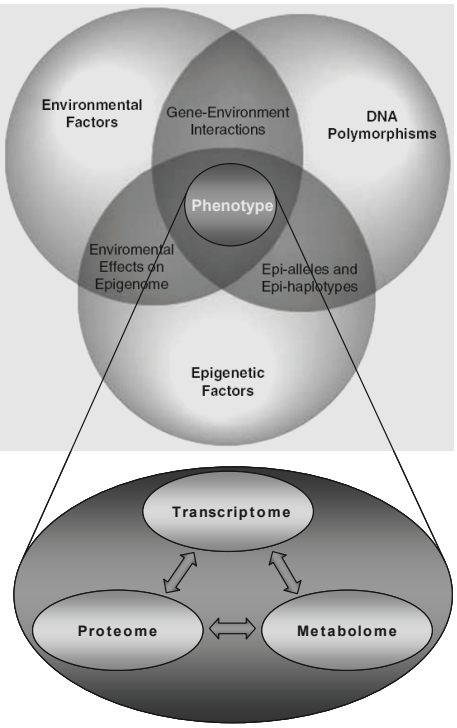
Biomarkers such as proteins, metabolites and cells of the immune system are being tested for their potential in early diagnosis of PDD. However, definitions of PDD and the ways of assigning them have changed throughout time making any correlation with biochemical markers difficult. Because PDD represents a spectrum of disorders with impairments in different areas that range from very mild sub clinical to severe clinical forms, it is inherent to PDD that their presence or absence in an individual can never be confirmed with 100% certainty. The question whether a diagnostic biomarker for PDD is feasible should not deal with the question whether symptom-based diagnosis is better than biomarker based diagnosis (i.e. is the biomarker the consequence of an established diagnostic system?), but merely with the fact whether a biomarker can identify those at risk of developing an impairment, which may be a part of a disorder named 'PDD', and whether these individuals are responsive to available interventions.

4. Etiology of schizophrenia and autism

The etiology of many psychiatric disorders is unknown. To determine which biomarkers (for prevention, diagnosis and treatment) are necessary, it is first important to consider what is known about the etiology. For example, current biomarker research will be less fruitful in establishing the biological causes of increased risk of schizophrenia in migrants, because the causes are likely to be very heterogeneous. Biomarker research should rather aim to identify and characterize groups or individuals with (epi-) genomes, transcriptomes, proteomes or

metabolomes that confer susceptibility to develop a mental disorder or that are responsive to available therapies. Integration of evolutionary medicine (see 'glossary'), epidemiology and life sciences is necessary to identify those individuals carrying susceptible '-omes'. For this we believe it is better to describe mental disorders as arbitrarily defined sets of symptoms that may have a hierarchical or dimensional (e.g. dimensions of positive, negative or general symptoms of schizophrenia) structure rather than as discrete categories [54]. We suggest that an extended model of Mills and Petronis [55] (**Figure 1**) best represents the components of modern complex disease and mental disorders, in particular.

Figure 1. ***Model of disease/phenotype.***
Adapted from [55]. Biomarker research and systems biology focus at molecular changes that determine the phenotype in response to the interplay of environmental factors, DNA polymorphisms and epigenetic factors. The etiology of disease, including that of mental disorders, can be found either in our hardware, i.e. DNA and highly heritable epigenetic imprints, or our software, i.e. the interplay between '-omes' and environmental and epigenetic factors, or both.



This section focuses on environmental risk factors, genetic susceptibility genes and epigenetic regulation in mental disorders to find the many, presumably overlapping, causes of mental disorders. **Table 1** shows a summary of the different risk factors putatively involved in the etiology of schizophrenia, and **Table 2** summarizes the risk factors implicated in the etiology of PDD.

Table 1. *Risk factors for schizophrenia.*

Psychological, Social and Cultural factors	*References*
Migration: migrants vs. native born (RR 2.9), 1st generation migrants (RR 2.7), 2nd generation (RR 4.5), dark-skinned migrants (RR 4.8)	[17;57]
Urbanicity: urban birth and urban childhood	[16;64]
Socioeconomic stress: socioeconomic deprivation at birth, social defeat, discrimination, acculturative stress	[35;64;74]
Childhood trauma: stressful events alter HPA axis and CRF system	[69;71]
Early life events	
Advanced paternal age: >35 years	[79]
Prenatal and perinatal risk factors (RR ±2.0): fetal growth retardation, fetal perinatal hypoxia, perinatal infections, perinatal stress, Rhesus incompatibility, and pre-pregnancy high and late-pregnancy low maternal BMI	[90;93;97;98]
Season of birth	[84;85]
Folate deficiency: periconception folate status, high 3rd trimester maternal homocysteine (Hcy) levels	[63;101;102]
Vitamin D deficiency	[61;62]
Genetics (RR 1.5-2.0)	
NRG1, DTNBP1, DAOA (G72), Tbx1, COMT, PRODH2, NPAS3, GRK4, DISC1,	[142-144]
Epigenetics	
RELN, GAD_{67}, MB-COMT	[153;155]

4.1. Psychological, Social and Cultural Risk Factors

4.1.1. Schizophrenia

Several factors such as migration, urbanicity and environmental stress have been associated with increased risk for schizophrenia [35] and other mental disorders. These risk factors seem to be strongly intertwined, because the many suggested causatives show considerable overlap and mutual modes of action. Unification of risk factors for different mental disorders may prove valuable in establishing common causes of closely-related mental disorders.

Table 2. *Risk factors for pervasive developmental disorders.*

Psychological, Social and Cultural factors	*References*
Migration: being born to mothers born outside Europe (adj. RR 1.4)	[76]
Urbanicity: high degree of urbanization of birthplace (adj. RR >1.6)	[76]
Early life events	
Advanced paternal and maternal age	[77]
Prenatal and perinatal complications (RR 1.5-2.5): low birth weight, short duration of gestation, obstetric complications associated with intrapartum hypoxia, Rhesus incompatibility	[77;94]
Prenatal and perinatal exposure to toxic substances: environmental pollutants, alcohol, tobacco, substances of abuse, medication	[125;126;128]
Genetics	
TSC1, TSC2, NF1, MECP2, SHANK3, SLC6A4, NBEA, PRKCB-I and -II, EN2, MET, *de novo* CNV	[146-149; 167;168]
Epigenetics	
BDNF, DLX5, UBE3A, GABR, RELN, FMR1	[146;152;153]

Migration does not seem to be generally associated with an increased risk for mood disorders [56] but this is not the case for schizophrenia. There is an increased risk of schizophrenia in migrants, notably 2nd generation (relative risk; RR 4.5) and dark-skinned migrants (RR 4.8), which is attributed to psychosocial factors including discrimination, social defeat and acculturative stress (i.e. stress caused by the psychological and social counter-part of cultural diffusion and admixture) as well as to biological factors such as folate and vitamin D deficiency [57]. The idea that experiences of psychosocial adversity by, possibly also genetically susceptible, individuals belonging to an ethnic minority increase their risk of developing schizophrenia fits findings of increased risk in white migrants [58] and non-black minority groups [59]. Furthermore, the high prevalence of PTSD (10%), major depression (5%) and general anxiety (4%) in refugees [60] emphasize the role of psychosocial and cultural factors. However, the vitamin D hypothesis of schizophrenia [61] is also attractive, because it explains many epidemiological issues of schizophrenia [62], for example, why 1st generation dark-skinned migrants and their offspring (2nd generation) are at increased risk. Next to vitamin D, qualitative and quantitative changes in the diet of carbon-1 (C_1) substrates (e.g. folic acid) in combination with polymorphisms in genes related to C_1-metabolism (e.g. the methylene tetrahydrofolate reductase (MTHFR) gene) are suspect of aberrant epigenetic control of DNA transcription in pregnancy resulting in increased

susceptibility for mental disorders [63]. Overall, one can conclude that although factors of psychosocial and cultural nature are important in the etiology of mental disorders, it seems that these risk factors are less easy targets for intervention and prevention than factors of dietary nature. E.g. nutritional fortification is more easily instituted than accomplishing a change in behavior in those that discriminate.

Urban birth or urban childhood compared to rural locations is suggested to be associated with an increased risk for schizophrenia. It explains around 30% of all schizophrenia incidence, thus being a major environmental risk factor [64]. Exposures to infectious agents, low prenatal vitamin D and folate levels [62], toxins associated with pollution and stress (social isolation) have been mentioned, although the real underlying cause remains to be elucidated [65]. Cognitive social capital, aspects of the degree of mutual trust, bonding and safety in neighborhoods are suggested to be important during the rearing of children and each of them modulates the risk of schizophrenia [64]. Selective migration to urban areas of individuals with proneness to schizophrenia has been explained by various factors related to poverty, the availability of services and easier access to cheap accommodation [17]. Remarkably, urbanicity as risk factor seems specific for the psychotic symptoms of schizophrenia and bipolar disorder, because affective illness of bipolar disorder was not shown to be associated with urbanicity [66;67]. While Williams et al. [25] found an increased prevalence rate of PDD in urban areas compared to rural/mixed areas; they attributed this finding to different diagnostic practices between locations. In addition, major depression was more prevalent in urban than rural areas if controlled for confounding factors like age, immigration status, race, working status and marital status [68]. Taken together, urbanicity is associated with an increased risk for at least some mental disorders. The biochemical background against which urbanicity acts in conjunction with other genetic and environmental risk factors will be difficult to draw.

Traumatic events, independent of migration and urbanicity, have been implicated in the etiology of schizophrenia by some [69], but not by others [70]. The relation between childhood trauma and depression, anxiety and panic disorders, PTSD, drug abuse and suicide attempts is much less debated [71]. A neurobiological explanation is sought in persistent, long-term effects of stressful events during a critical period of development on respectively the activity and sensitivity of the corticotropin-releasing factor (CRF) system as well as the hypothalamic-pituitary-adrenal (HPA) axis [71]. These systems are important in the mediation of mood and anxiety symptoms. For example, the CRF system shows increased activity in patients with depressive symptoms [72], while depressed abused children compared to controls or to depressed non-abused children showed increased corticotropin (ACTH) excretion post CRF administration [73]. Altogether,

childhood trauma may be one of the many environmental factors lowering the threshold to develop a mental disorder in those individuals that are (epi-) genetically more susceptible.

4.1.2. Pervasive Developmental Disorders

Risk factors of psychosocial and cultural nature for PDD are less easily identified, because the etiological role of these factors may prove less distinct. Kanner emphasized in 1943 the unusually high educational background and professional achievements of parents [45] of children with autism but the association between autism and socioeconomic class could not be confirmed [22]. In contrast to PDD, socioeconomic deprivation at birth at the level of the individual and community is associated with increased risk of schizophrenia [74] and another recent study implied that socioeconomic status partially explains the observed higher risk of schizophrenia in African Americans compared to white Americans [75]. A recent study in Denmark [76], investigating familial risk factors for autism, identified having an affected sibling (adjusted RR 22.3), maternal history of psychiatric disorder (adj. RR 2.0), a high degree of urbanization of birthplace (adj. RR > 1.6) and being born to mothers born outside Europe (adj. RR 1.4). The latter factor, i.e. maternal migration, is explained by missed immunizations in the new country of residence and/or selective migration of people with a genetic vulnerability to autism [77]. We believe that similar factors underlying the association between migration and urbanicity and schizophrenia, also apply to PDD, because the etiology of several psychiatric disorders might not be so different.

Summarizing, the available literature supports an important role of psychosocial and cultural risk factors in the development of mental disorders. It seems that many of the psychosocial and/or environmental factors, such as urban location, can be termed “mental stress factors”, which challenge the human brain/organism more than it was meant to deal with. Susceptible individuals probably are unable to cope, or cope differently, with this stress and are experienced by others as ‘crazy’. Obviously, identification of key risk factors is important, because it allows us to develop selective or universal preventive measures and targeted interventions. However, the complexity and interaction of the underlying causes may thwart a clear definition of these targets.

4.2. Early-life events

There is reasonable evidence to believe that the origin of many disorders, including that of mental disorders, can be found *in utero*. In analogy to the Barker hypothesis [78], which links fetal malnutrition via abnormal metabolic programming to

cardiovascular disease in later life, perinatal factors disrupting brain development *in utero* may confer increased vulnerability to develop a mental disorder. Suboptimal neurodevelopment may only be partially reparable during post-natal life. In this section we will discuss several early-life events that may be deleterious for cognitive and mental functioning in later life.

Advanced parental age has been implicated in many mental disorders. In autism, advanced paternal and maternal age, were consistently shown to be associated with an increased risk of having a child with ASD [77] and similar results were obtained from a meta-analysis of the effects of paternal age for schizophrenia risk [79]. At the time of conception, the fertilized egg has undergone a maximum of 23 replications, whereas for sperm cells this number varies non-linearly with age from 35 to 840 at the age of 15 and 50 years, respectively [80], with each replication carrying a chance of mutation in the germ-line. Interestingly, paternal and also maternal age independently have an effect on offspring IQ-scores, with increasing maternal age affecting fetal neurodevelopment through age-related alterations in the *in-utero* environment [81]. Paternal *de novo* mutations and/or altered genetic imprinting [82] and maternal nucleotide repeat instability [83] have been proposed as mechanisms.

The season of birth effect on mental disorders has been most consistently shown for schizophrenia [84;85]. Winter/early spring birth in the Northern hemisphere and births 3-4 months after rain season in northern Brazil [86] are associated with an increased risk of schizophrenia. However, winter/spring births are also associated with superior outcomes with respect to physical and cognitive development in the general population, suggesting an impact of season of birth on developmental trajectories [87]. Remarkably, the season of birth effect was not observed in neurodevelopmental disorders such as ASD, hyperkinetic disorder, Tourette syndrome and obsessive-compulsive disorder (OCD), although in this study such an effect could not be ruled out [88]. Significant seasonal effects on dietary intake of micro- and macronutrients, including fat, carbohydrate, vitamin C and D, and B-vitamins during pregnancy has been proposed as explanation for the season of birth effect on cardiovascular diseases and mental disorders [89]. In addition, McGrath et al. [87] suggested differential exposure to the complex of interacting downstream consequences of biometeorological variables (temperature, rainfall, UV-radiation etc.), including those that have an impact on health status, energy expenditure, disease exposure, vitamin D status etc. at critical periods of development *in utero*, to be responsible for the counterintuitive result of the association of winter/spring births with both better physical and cognitive outcomes in the general population and with an increased risk for schizophrenia.

Prenatal and perinatal complications are frequently associated with increased risk for mental disorders, including autism (RR 1.5-2.5) [77], schizophrenia (RR ±2.0) [90] and eating disorders [91]. For bipolar disorder, however, no robust evidence for such an association is present [92]. Low birth weight (LBW), short duration of gestation and obstetric complications associated with intrapartum hypoxia were most consistently found to increase the risk of autism [77]. Fetal growth retardation, fetal perinatal hypoxia and other prenatal risk factors such as infections, medication, stress, nutritional deficiency and Rhesus incompatibility, were found to increase the risk of schizophrenia [93], while growth restriction and newborn hypoxia were found to be risk factors for autism [77]. Smoking, the use of contraceptives at time of conception and Rhesus incompatibility during pregnancy were also found to be associated with an increased risk for autism [94]. There seems to be a consistent association with adverse events during pregnancy, at delivery and during the neonatal phase with the development of at least some mental disorders in early or later life. Perhaps these adverse events are early expressions of the presence of a risk factor, like a susceptible (epi-)genotype that predisposes to the development of obstetrical complications in the mother and mental disorders in the offspring.

Likely, low weight at birth (LBW) (<2500 g) and fetal growth restriction can be considered to be indicators of a heterogeneous set of adverse intrauterine effects. However, both LBW as well as fetal growth restriction are associated with preterm birth. Black race, maternal thinness (BMI<20), a history of a prior preterm birth, a short cervical length and a positive test result for cervical or vaginal fluid fetal fibronectin were identified as strong predictors of preterm birth in the U.S. [95]. Also, intrauterine bacterial infections are thought to be responsible for 85% of spontaneous preterm births [96], especially malaria and HIV in the developing countries. Interestingly, high maternal BMI seems to protect against two risk factors for growth restriction which are maternal smoking, and maternal stress, but interventions directed at increasing birth weight seemed only effective in thin women [96]. Nevertheless, both late-pregnancy low [97] and pre-pregnancy high maternal BMI [98] have been linked to increased risk for schizophrenia in the offspring. Low maternal BMI may mediate its effects by similar mechanisms that are implicated in a two-fold higher risk of having offspring with schizophrenia in women that experience starvation during early pregnancy [99;100]. Low periconception folate status [101] and, conceivably associated, high 3rd trimester maternal homocysteine (Hcy) levels [102] have been implicated as key nutritional mediators of the adverse effects of famine during early pregnancy. The many possible effects of folate and homocysteine on schizophrenia are discussed in detail in [63]. However, elevated 3rd trimester Hcy levels have been suggested to cause

subtle damage to placental vasculature thus compromising oxygen delivery to the fetus [102]. It is likely that besides many factors that adversely affect the fetus' growth, psychosocial factors are important for risk-increasing obstetric events themselves. The quest for a single risk factor that accounts for the effects of LBW and growth restriction on the development of mental disorders should thus be discouraged.

Prolonged or acute oxygen deprivation to the fetus may be a major risk factor for neuropsychological and neuropsychiatric disturbances. Cerebral hypoxia-ischemia harms fetal brain development considerably, but timing and severity determine the outcome in terms of the severity of the damage and the regions of the brain affected [103]. Energy depletion and subsequent generation of reactive-oxygen species are primarily responsible for hypoxia-associated neuronal cell death [103]. The origins of fetal hypoxia are heterogeneous and may include, in addition to overt fetal distress, maternal hypertension (e.g. caused by pre-eclampsia), gestational diabetes mellitus (DM), hemolytic diseases (e.g. Rhesus incompatibility), cord encircling of the neck and prolonged labor [77;104]. The suggestion that genetic vulnerability for schizophrenia [105], autism and other mental disorders increases obstetric sub optimality, is intriguing and warrants further research [77;106]. This relation between genotype and obstetrics would mean that obstetric events are early indicators of susceptibility genes present in individuals at risk of developing a mental disorder.

Several reports have suggested that prenatal exposure to bacterial and viral infections contributes to the etiology of schizophrenia (reviewed in [107]), autism [108;109] and mental retardation [110]. Prenatal infections with polio, rubella, influenza and toxoplasmosis have been associated with schizophrenia. In contrast to the 5-10% increase in risk of schizophrenia through second trimester exposure to the poliovirus [111], prenatal exposure to rubella, a well-known central nervous system teratogen, is associated with a 10-20 fold increase in risk of schizophrenia [112] and with an increased risk of autism [108]. First trimester exposure to influenza confers a 7-fold risk, while early to mid gestation exposure was associated with a 3-fold risk of developing schizophrenia [113]. A 2.5-fold increase of risk for schizophrenia was noted to be associated with elevated maternal IgG antibodies against *Toxoplasma gondii*, a ubiquitous intracellular parasite [114]. Interestingly, a recent report by Cetinkaya et al. [115] found that two third of patients with schizophrenia had elevated serum levels of anti *Toxoplasma gondii* antibodies compared to 20-25% of depressed patients and controls. Second trimester increases of maternal cytokine levels, including interleukin 8 (IL-8), are also significantly associated with pregnancies giving rise to schizophrenia cases [116]. It is important to note that the results from studies of prenatal infection and schizophrenia might

have public health implications, because there are many possible preventive strategies for bacterial and viral infections.

For autism, exposure of the fetus to the cytomegalovirus (CMV) in the 3rd trimester has been implicated in the etiology of some children with autistic-like behavior [109]. Other congenital infections have been implicated in autism, but evidence is less convincing for measles, mumps, varicella and intrauterine human parvovirus [117]. Congenital rubella [118] and herpes simplex [119] seem more consistently associated with autism, mental retardation and behavioral pathology. Prenatal infection with CMV [110] or *Toxoplasma gondii* [120] is also suggested to increase the risk of mental retardation. Altogether, prenatal bacterial or viral infections seem to disrupt normal neurodevelopment with neuropsychiatric consequences in later life, suggesting that the downstream consequence (e.g. cytokine release) of the inflammatory process rather than the inflammatory agent itself (e.g. lipopolysaccharides [121]) is responsible for abnormal neurodevelopment.

A possible explanation for the mechanism by which maternal infection affects embryogenesis is through hypoxia, hyperthermia, malnutrition, and the effect of elevated levels of circulating cytokines on gene expression in the brain. Fatemi et al. [122] showed altered regulation of a subset of genes in brains of mouse offspring exposed to prenatal infection. This potentially leads to permanent changes in brain structure and function. Cytokines, probably of maternal, placental and fetal origin, might suppress expression of genes resulting in subsequent reduced protein availability [123]. Reelin [124], for example, is an important glycoprotein involved in guidance of neuronal and glial cells during embryogenesis, and reduced expression of the reelin-gene through mutation or promoter hypermethylation has been shown to result in cognitive deficits that are similar to those that are often observed in autism and schizophrenia. It is hypothesized that disrupted neurodevelopment, rather than neurodegeneration, is associated with prenatal infection and central to the etiopathogenesis and disease process of schizophrenia and autism [125-127].

Environmental exposure to toxic substances in fetal life such as alcohol, substances of abuse, heavy-metals (lead, methylmercury, arsenic), dry-cleaning agents (tetrachloroethylene), toluene, PCBs, certain classes of medication, and many other industrial chemicals are implicated in the etiology of, mainly, neurodevelopmental disorders [128]. Exposure of the embryo to ethanol is associated with increased risk of the Fetal Alcohol Syndrome (FAS) and its less severe variants known as Fetal Alcohol Effects (FAE). Although congenital anomalies that are usually observed in children with FAS are often not seen in children with autism, considerable overlap in behavioral pathology suggests a

common factor. Therefore, the complex cognitive, behavioral, and physical symptomatology in children with FAS [129] may frequently be misdiagnosed as PDD, for psychological and political reasons that will not be discussed in this review. Prenatal cocaine exposure (PCE) has, however, not been linked with congenital anomalies or medical complications, but it has been found to be an independent risk factor for behavioral problems at school age [130]. Remarkable, this study [130] showed that the combined effects of prenatal and postnatal exposure to tobacco and alcohol on childhood behavior were found to be greater than that of PCE. Use of thalidomide, misoprostol and valproic acid during the 1st trimester, notably during the first 8 weeks of embryogenesis, is associated with autism [126;131], while the use of analgesics during the 2nd trimester [132] and of diuretics for treatment of maternal hypertension in the 3rd trimester [133] are thought to play a role in the etiology schizophrenia. The neurodevelopmental and neurotoxic effects of some industrial chemicals are diverse and a universal mode of action will be hard to find. Disturbance of thyroid function and subsequent harm to the embryo and fetus has been proposed [134]. The different effects, ranging from minor anomalies to malformations, resulting from short-term (1-3 weeks) exposure to the aforementioned exogenous substances during the first trimester, suggest that each of these agents strikes during a critical phase ("vulnerability window") during the embryogenic period, with physical and neuropsychiatric consequences at birth and thereafter. Unfortunately, such insults are often permanent, because there is little potential for later repair. It is imaginable that presence of a susceptible genotype, i.e. a maternal and/or fetal genotype less able to detoxify, can moderate the effects of environmental toxicity. Studies (in animals) into the teratogenic effects of toxic agents may thus benefit from complementary biomarker-related research.

Other life events that are implicated in the etiology of mental disorders are brain trauma and substance abuse. For autism, e.g., a case-series study of patients with PDD in Tanzania with severe malaria and subsequent recovery during the first years of life showed an immediate onset of autistic-like behavior in some patients, which had entirely normal development before their malaria-infection [135]. Tourette syndrome and tics have also been associated with insults to the basal ganglia, such as head trauma, encephalitis and other causes [136]. While substance abuse, e.g. amphetamine (dopamine D_2-receptor), ketamine (N-methyl-D-aspartic acid [NMDA] receptor), phencyclidine (PCP), LSD, heroin, cocaine, can merge into psychosis-like symptoms, the diagnosis of schizophrenia is generally not established in this group of patients. However, substance abusers tend to be overrepresented among patients with mental disorders, advancing a common vulnerability [137].

It is also rewarding to investigate potential links between somatic diseases and psychiatric symptoms to obtain better insight into cause-symptom relations. For example, adults with celiac disease have a high prevalence of depressive symptoms [138]. It seems that these patients are at increased risk for non-affective psychosis [139] and are more likely to have ADHD-like symptomatology [140]. Primary hyperparathyroidism (PHPT), characterized by thirst, fractures, osteoporosis, urolithiasis, is also associated with psychosomatic symptoms that are usually encountered in obsessive-compulsive disorders, depression, anxiety, and paranoia [141]. The occurrence of psychiatric symptoms in somatic disease, and parallels between mental disorders and somatic disorders, may provide new insights into disease-mechanisms and yield valuable ideas regarding new therapies.

In summary, early life events ranging from those that affect the quality of germ-line cells to those that disrupt neurological and cognitive function later in life, and all adverse events in between, have been implicated in the etiology of mental disorders. On one hand this large number of identified risk factors emphasizes the speed of science, but the fact that only few risk factors have been linked to a proven mechanism shows that there is still a large gap in our knowledge on the other hand. Integrative approaches, studies using extensively-characterized population cohorts, longitudinally acquired bio-repositories and life-long follow-up, performed on different continents are only one part of the answer. Intervention/prevention-based medicine might be the other part. Of course, this research can be supported and complemented by biomarker research, elucidating disease-mechanisms, prognostic and diagnostic phenomena and supporting tailored interventions.

4.3. Genetics and epigenetics of mental disorders

There has been a great focus on the genetic and, recently, epigenetic basis of mental disorders. Evidence for a genetic contribution in the etiology of schizophrenia [142-144], bipolar disorder and major depression [143;145], and autism [146-149] has been comprehensively reviewed. The concept of a strongly genetic contribution in the etiology of mental disorders is fuelled by a body of studies in twins and affected families. A major problem with genetic research is the assumption that mental disorders have a predominantly genetic basis, which underestimates the contribution of environmental factors. The assumption appears incorrect because of large effect-sizes of some environmental factors, e.g. 10-20 fold higher risk of schizophrenia associated with prenatal rubella exposure. In addition, the value of conclusions from twin-studies is questioned because these studies of MZ or DZ twin reared-together or reared-apart, may not measure anything more than environmental influences, error, and bias [150]. Interestingly, during their lifetime

twins increasingly differ in their epigenotype, resulting in different phenotypes and different susceptibility for diseases like mental disorders [151]. The role of epigenetic mechanisms in psychiatric disorders is also increasingly recognized [146;152-155].

To understand the mechanisms by which predisposing genes interact with the environment to result in the precipitation of a mental disorder, we will review literature regarding the role of genetics and epigenetics in the etiology of mental disorders. This section, however, will not exhaustively discuss all chromosomes, loci, alleles, and genes etc. that have ever been implied in schizophrenia, bipolar disorder, major depression and autism. Instead, some current achievements and failures as well as difficulties and new approaches in genetic analysis of psychiatric disorders will be reviewed. In addition, their value in diagnostics and treatment will be discussed.

4.3.1. Genetics of mental disorders

It seems a paradox that susceptibility alleles that confer an increased risk for developing a mental disorder are still among us, notably when mental disorders are considered as common, harmful and heritable. Some believe this to be the result of a polygenic mutation-selection balance [156], i.e. that our complex genotype, which is required for our underlying human behavioral phenotype is inevitably prone to suffer to some extent from mutations. This idea is supported by the notion that mental disorders do not inherit by Mendel's law. The majority of the scientific community is in favor of a multi-loci (polygenic) mode of inheritance of mental disorders. Other explanations for the presence of susceptibility genes throughout the millions of years of evolution are based on ancestral neutrality (mismatch between ancestral and current environments) and on balance-selection (adaptive benefits). The different models are commented by Keller and Miller [156], and it might be important to view the genetic etiology of schizophrenia and autism and other mental disorders against this background.

Two approaches are being adopted in the search for mental disorder genes: linkage studies and association studies. Linkage studies aim to select candidate gene loci by genome-wide or local gene mapping in families and by defining the tendency for alleles closely spaced on the same chromosome and coinciding with the phenotype of interest to be transmitted from one generation to another as an intact unit through meiosis. The next step is generally to examine candidate genes by means of association studies. Most molecular genetic studies on mental disorders follow the common disease common variant (CDCV) model, which implies the study of relatively common polymorphism that confer susceptibility to common

diseases like heart disease. However, by doing so rare causative variants may be overlooked.

Unfortunately, unclear diagnostic boundaries, multiloci-type inheritance, epistasis (gene-gene interactions), clinical and etiologic heterogeneity and confounding gene-environment and environment-gene interactions have impeded linkage and association studies considerably leading to many inconclusive and non-replicable findings [142]. More stringent criteria for publication of results from association studies are under way [157] to avoid future statements like "to date, no causative gene or genetic risk factor has been identified for bipolar disorder or depression" [145] and "no gene has been proven to not be an autism disease gene" [158].

While the genetic causes of mental disorders have not been uncovered yet, parallels should also be sought in the ways the etiology and pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and Huntington's disease are investigated. It is important (i) to understand rare familial variants, (ii) to identify (interacting) causative genes to define pathogenic pathways and therapeutic targets, (iii) to investigate commonalities among different disorders, (iv) to identify mutations that increase the risk of developing a disease but that themselves are not causally related, and (v) to consider the neurotoxicity of RNAs and proteins (e.g. in Huntington's disease [159]) deriving from genetic changes such as point or frameshift mutations or deletions [144]. For schizophrenia, which is increasingly viewed as a subtle disorder of neurodevelopment, clues to the etiology and pathogenesis may be provided by severe disorders of cortical development like lissencephalies [144]. For example, an established cause of lissencephaly is mutation of the RELN-gene, which codes for reelin. Reelin has a key role in cortical neuronal migration [124] and reduced expression of RELN in post-mortem brains of individuals with schizophrenia and bipolar disorder has been reported [160]. Moreover, neuropathological investigations of schizophrenia point more to a neurodevelopmental abnormality than a neurodegenerative disease [161]. Most genes that have been implicated in mental disorders have a key role in neurodevelopment and/or neurotransmission.

4.3.1.1. Genetics of schizophrenia [142-144]

The 10-times higher LMR in relatives of patients with schizophrenia [162], the higher concordance rates in monozygotic (MZ, ~50%) than dizygotic (DZ, ~17%) twins [163] have led to an estimate of the heritability of tendency to develop schizophrenia of about 80%. Gene-environment interactions certainly contribute to the overall risk, emphasizing the idea that schizophrenia may be genetically

mediated but not determined [142]. Interestingly, the relative risks for schizophrenia at the loci identified so far are rather low (range 1.5-2.0) indicating modest effect sizes [144].

Regions 1q, 5q, 6p, 6q, 8p, 10p, 13q, 15q and 22q have been confirmed in more than one linkage study, and association replication has been observed for neuregulin 1 (NRG1) and dystrobrevin binding protein I (dysbindin; DTNBP1). Other genes that have, though not consistently, been associated with schizophrenia are D-amino acid oxidase [DAO] activator (DAOA; formerly called G72) and genes located on chromosome 22, catechol-O-methyl transferase (COMT) and proline dehydrogenase 2 (PRODH2).

Neuregulin 1 was identified by fine mapping of chromosome 8p and is believed to code for multiple protein products that are implicated in neuronal differentiation and migration as well as in expression and activation of neurotransmitter receptors like the NMDA-receptor. Polymorphisms leading to alternative splice variants of NRG1 may lead to proteins with enhanced or reduced function. Dysbindin was shown to be associated with schizophrenia through chromosome 6p linkage [142]. Its pre- and postsynaptic presence, its suggested role in glutaminergic neurotransmission, and its reduced expression at both RNA and protein levels in certain brain regions of schizophrenia patients have fostered the search for dysbindin polymorphisms. Dysbindin seems to interact with serine-threonine kinase 1 (AKT1), a protein that is involved in growth-factor induced neuronal survival. The AKT1 gene has recently been shown to be associated with schizophrenia [164]. D-amino acid oxidase is activated by DAOA which oxidizes D-serine, a coagonist at the "glycine site" of the NMDA receptor. The potential therapeutic efficacy of D-serine and reports of reduced blood and cerebrospinal fluid (CSF) D-serine levels in individuals with schizophrenia warrant further investigation of the DAOA-system. Most replication studies have confirmed a role of DAOA in schizophrenia.

Twenty to 30% of patients with the chromosomal microdeletion syndrome (velo-cardio-facial syndrome; a deletion in chromosome 22q11 that encompasses appr. 27 genes) have schizophrenia or another major psychiatric disorder with psychosis-like symptoms. Among the deleted genes is T-box 1 (Tbx1; transcription factor involved in regulation of otocyst development), which is expressed in the microvasculature of the brain. The COMT gene is also coded on chromosome 22q11 and has received more attention, because the enzyme participates in the clearance of catecholamines, including dopamine, from the synapses. Replacement of methionine by valine at codon 108 (soluble form; S-COMT) or codon 158 (membrane-bound form; MB-COMT) has been suggested to decrease COMT-activity, thus resulting in prolonged elevated levels of dopamine in critical central

synaptic clefts. However, reduced COMT-activity appears to be more strongly associated with cognitive function than with schizophrenia. Altered PRODH2 activity influencing the availability of glutamate, has also been linked to deletions in the 22q11 chromosome region. The only two reports considering chromosomal translocations in individuals with schizophrenia deal with neuronal PAS domain protein 3 (NPAS3) and G-protein receptor kinase 4 (GRK4), which code for a transcriptional regulatory factor and glutamate kainate receptor, respectively.

DISC1 (disrupted in schizophrenia 1), is emerging as the best supported candidate gene for schizophrenia. It has been identified via a balanced (1:11) chromosomal translocation, segregating with schizophrenia, bipolar disorder and other mental illnesses. Affected individuals have either schizophrenia or an affective disorder. Also, endophenotypical reduced P300 amplitude and latency linked to schizophrenia, was associated with translocation in both unaffected as well as affected carriers. On a structural/functional level, DISC1 haplotypes are associated with alterations in hippocampal function, fMRI signals, working memory and cognition. Although it is presently unclear whether haploinsufficiency or dominant-negative interactions mediate DISC1 loss-of-function, elucidation of normal DISC1 function is critical for understanding DISC1-related diseases. DISC1 is distributed throughout neurons, nuclei, mitochondria and neurites and its function is linked to neural development via neuronal migration, neurite outgrowth and neuronal maturation, and to neural functioning via modulation of cytoskeleton function, synaptic transmission and plasticity. DISC1 protein interacts with many other proteins to form complexes that are vital for proper neurodevelopment and neural functioning.

The interactivity of DISC1 is suggestive of the idea that multiple subtle dysfunctional genes and/or gene-products can lead to endophenotypes associated with several mental disorders such as schizophrenia, bipolar disorder and depression. Also, the interactivity-requirement supports the notion that the functionality of a protein complex can be adversely affected if a single protein constituent is reduced in quality or quantity. For example, DISC1 interacts with phosphodiesterase 4B to regulate neuronal cAMP cell signaling. Thus, if such a protein-complex is involved in critical processes of brain development, brain functioning and behavior, it can have differential down-stream consequences. Other processes, of which the functionality is subject to genetic variation, may increase or repair the damage, thus adding to the importance of epistasis in mental disorders. Taken together, genes associated with schizophrenia probably have different etiologic and pathogenic effects, each affecting particular neurobiological processes to different extents, in turn causing specific phenotypes.

4.3.1.2. Genetics of bipolar disorder and unipolar depression [143;145]

Familial aggregation is higher for bipolar disorder than for unipolar depression as shown by higher rates of sibling recurrence risk (5-10% vs. 2.5-3.5%) and MZ-twin concordance (45-70% vs. 40-50%). The heritability estimate for bipolar disorder (80-90%) is higher than for unipolar depression (33-42%), supporting a stronger environmental contribution in unipolar depression [143]. The genetic contribution to the etiology of bipolar disorder resembles that of schizophrenia, suggesting a common genetic background. Results from family and twin linkage studies show convergent or overlapping clinical features and susceptibility genes (DAOA, DISC1, NRG1 and COMT) for schizophrenia, schizoaffective disorder and bipolar disorder [165].

Linkage studies in bipolar disorder resulted in suspected loci at 2p, 4p, 4q, 6q, 8q, 9p, 10q, 12q, 11p, 13q, 14q, 16p, 16q, 18q, 21q, 22q and Xq [145]. Unfortunately, results from these linkage studies and subsequent association studies of specific gene loci have not provided unambiguously identified susceptibility genes for bipolar disorder or depression. Functional genetic studies in bipolar disorder have focused on neurotransmission cascades that are also implicated in schizophrenia: the monoamine pathway (dopamine, serotonin [5-HT] and noradrenaline), intracellular signaling systems, the GABA (γ-aminobutyric acid)ergic system, proto-cadherin and genes encoding for other targets of mood-stabilizers such as lithium. Although effect-sizes were modest (RR < 2), functional polymorphisms for monoamine-oxidase-A (MAO-A), COMT and the 5-HT transporter (5-HTT) genes have been implicated. However, these positive results still await replication in independent samples of sufficient size.

For DAOA (13q34), which is also implicated in schizophrenia, no pathologically relevant variant has been identified despite independent confirmation of its variation influencing susceptibility to bipolar disorder. In view of these findings the DAO containing region 12q23, which is implicated in both bipolar disorder as well as unipolar disorder, warrants more thorough study. Another interesting candidate gene is brain-derived neutrophic factor (BDNF). BDNF (putatively located on 11p13), a neurotrophin, has a role in promoting and modifying growth, development and survival of neuronal populations and, in the mature nervous system, it is involved in activity-dependent neuronal plasticity. The common functional Val66Met polymorphism of BDNF is thought to be associated with susceptibility to a specific aspect of the clinical bipolar phenotype, rather than influencing susceptibility to bipolar disorder as a whole. G-protein receptor kinase 3 (GRK3) and XBP1 (encodes a transcription factor that regulates MHC class II genes) (both 22q) have also been associated with bipolar disorder through

circumstantial evidence coming from a rodent model of mania and an effect of the functional polymorphism on mood stabilizer action, respectively. Many other genes have been implicated in bipolar disorder and of these, association of DAOA is most robust [145]. Some studies have increased the meaningfulness of the association by being able to relate gene polymorphisms to persecutory delusion or psychosis, but these findings could not be replicated.

Compared to bipolar disorder, unipolar depression is more heterogeneous and there is evidence for a large environmental component in its etiology. Large sample sizes and correction for confounding environmental factors are thus required. It is not surprising that few consistent positive findings of genetic studies concerning depression have been published. The 12q22-23 region is the only region that has been linked to unipolar depression, and also to anxiety traits. Gender-specificity in linkage signals has been observed, but awaits replication. Functional polymorphisms of 5-HTT, tryptophan hydroxylase 2 (TPH2) and BDNF have often been studied in combination with environmental factors to explain the onset of depression. Most interesting is the interaction between polymorph 5-HTT and occurrence of life events in early childhood. A rare loss-of-function mutation in TPH2, a brain-specific enzyme involved in 5-HT synthesis, was found in approximately 10% of patients with major depression, but this finding was not replicated in four subsequent studies. Despite initial positive findings, many subsequent studies have not been able to associate 5-HTT, BDNF and COMT with depression, possibly due to poor study design or false-positive initial findings.

More robust strategies identifying genetic risk factors or causative genes for mood disorders are required. In addition, researchers may consider experimenting with psychiatric nosologies other than DSM-IV and ICD-10, because the Kraepelin dichotomy that distinguishes schizophrenia and bipolar affective disorder as distinct entities with separate underlying disease processes and treatments seems outdated [165]. Further, the idea of convergent or overlapping susceptibility genes leading to a spectrum of disorders ranging from bipolar disorder to schizophrenia supports the view of Keller and Miller of a polygenetic mutation-selection model for mental disorders [156].

4.3.1.3. Genetics of pervasive developmental disorders [146-149]

Concordance of the narrow phenotype of ASD in monozygotic twins is 82-92%, while this is only 1-10% for dizygotic twins. Sibling recurrence risk is estimated at 2-3% and heritability is estimated to be >90%. Social and non-social autistic traits seem highly, but independently, genetically determined in ASD. Sub-threshold autistic traits are also more frequently present in siblings and parents of individuals with ASD. Together, these findings have incited researchers to believe that autism

is one of the most genetically determined neuropsychiatric disorders. However, the finding of concordance rates below 100% points to a weak but definitely significant influence of environmental factors on the ASD phenotype and the value of results coming from twin-studies should be reconsidered [150]. Gene-environment interactions, the high degree of genetic heterogeneity, the polygenic or oligenic mode of inheritance, and significant epistasis have complicated the search for autism-genes significantly.

Cytogenetic, linkage, candidate gene association, and recently also *de novo* copy number variation (CNV) studies are used to identify ASD susceptibility genes. The former kind has yielded valuable information through the study of 'syndromic' autism. About 10-15% of autism is syndromic, which means that the autism is secondary to a genetic disorder such as the chromosomal rearrangement syndromes Angelman and Prader-Willi (PWS), fragile X syndrome, tuberous sclerosis and neurofibromatosis, or the result of exposure to teratogenic agents. For the majority of autism cases (85-90%) the genetic origin is unknown. However, the cases in which autism is syndromic are especially relevant with regard to prevention of ASD by genetic counseling. Moreover, the syndromic cases present an opportunity for pinpointing the underlying genetic abnormalities and for investigating parallels with non-syndromic cases.

The rate of cytogenetic abnormalities in autistic disorder is estimated to be 3-5%. Duplication or inversion of the chromosomal 15q11-13 region, e.g., has a prevalence rate of about 1% in ASD and this region is also related to other developmental and behavioral syndromes. Deletions of the maternal or paternal chromosome 15q11-13 region are associated with the Angelman syndrome and PWS, respectively. Several genetic mechanisms have been implicated in Angelman, such as interstitial deletion of a maternal chromosome (70-75%), mutation of the ubiquitin-protein ligase E3A (UBE3A) gene or mutation in the imprinting center (20%), abnormal methylation (3-5%), and paternal uniparental disomy (UPD) in combination with the lack of a maternal copy (2-3%). Angelman syndrome has many characteristics in common with ASD, including moderate to severe mental retardation, absence of language development and motor stereotypies. For PWS similar mechanisms have been proposed such as interstitial deletion of the maternal chromosome (70-80%), maternal UPD in combination with the lack of a paternal copy (20-30%), and mutation in the imprinting center (1-2%). PWS subjects with UPD tend to have autistic-like impairment in social interaction.

The other syndromic cases are associated with single-gene mutations of the following genes: fragile-X mental retardation 1 (FMR1), tuberous sclerosis 1 (TSC1), tuberous sclerosis 2 (TSC2), neurofibromatosis 1 (NF1) and MECP2. More rare but treatable syndromes associated with ASD are phenylketonuria (PKU) and the

Smith-Lemli-Opitz syndrome. PKU as a cause of ASD has become rare in countries with an established neonatal screening program. Individuals with the Smith-Lemli-Opitz syndrome, which is associated with increased serum 7-dehydrocholesterol and syndactyly (webbing of fingers or toes) of toes 2 and 3, can be treated with a high-cholesterol diet. The X-linked genes MECP2 and FMR1 are involved in autism secondary to the Rett and the fragile X syndrome, respectively. MECP2 is a methylated-DNA binding protein that regulates gene expression through chromatin remodeling. MECP2-activity is thought to be important for synapse maintenance and remodeling because of the regressive nature of Rett. FMR1-gene silencing by promoter hypermethylation and subsequent reduced translation of FMR protein, which is involved in mRNA transport, results in fragile X syndrome (2-5% of individuals with ASD), a common cause of mild to moderate mental retardation in boys. The FMR protein function is modulated by GTPase activity which is crucial for control of cytoskeletal dynamics. Next to GTPase activating proteins, guanosine exchange factors are involved in ASD and mental retardation.

Mutations in tumor-suppressor genes (TSC1 and TSC2) related to tuberous sclerosis may be responsible for the localization of tubers to the temporal cortex generally observed in ASD. Tuberous sclerosis is an autosomal-dominant neurocutaneous disorder, which is 100-times more prevalent in ASD. Neurofibromatosis type 1 is a disease in which the growth properties of neural-crest derived cells are affected. It is caused by mutation in NF1 (encodes neurofibromin which is a tumor suppressor protein) and is associated with features such as toe syndactyly, cutaneous malformations and mental retardation. Despite fine-mapping of the X-chromosome, which is driven by the marked sex-difference in ASD, most linkage study outcomes are negative. However, linkage studies have shown 7q, 16p and 17q to be linked to ASD in male only pairs. Studies on maternally and paternally imprinted loci are, to date, inconclusive.

Unfortunately, chromosomal abnormality studies have not led to the identification of a common autism risk allele yet. Combining results from studies to rare non-syndromic chromosomal abnormalities in families of ASD subjects with those from candidate genes that reside in suspected chromosomal regions, have indicated a role for UBE3A and GABA-A receptor (GABR) β3, both mapped to 15q11-13, and the neuroligin (NLGN) gene family. The five NLGN-genes are X-linked and encode cell adhesion molecules localized at glutaminergic synapses or GABAergic synapses. They are thought to play a crucial role in synapse formation and their association with scaffolding proteins seemingly regulates the glutamate-GABA balance, possibly explaining the high prevalence of epilepsy in ASD. The above findings exemplify that identification of rare variants may have significant

value and investigations of phenomena of phenotypic overlap is of fundamental importance.

At the synapse, appropriate connectivity between cytoskeleton and membrane proteins is mediated by scaffolding proteins, such as encoded by the SH3 and multiple ankyrin repeat domains (SHANK3) gene, which are crucial for dendritic morphology. SHANK3 is also a binding partner for neuroligins, which is suggestive for a role of SHANK3 in the NLGN-pathway of autism. This pathway connects actin cytoskeleton to the postsynaptic scaffold at glutaminergic synapses. Variants of genes encoding neurotransmitter receptors and transporters might also confer susceptibility to or modulate ASD-associated behavior.

The most studied gene is the SLC6A4-gene, which encodes 5-HTT. However, SLC6A4 gene variants seem to have small effects on blood 5-HT levels in ASD. Functional polymorphism of 5-HTT have also been implicated in stereotyped behavior. The second most genotyped neurotransmission-genes are those of the GABA-receptor cluster. Significant epistasis of GABR α4 – GABR β1 has been reported. This is especially interesting, because of the involvement of the GABA-ergic systems in seizures in ASD. Glutaminergic receptor genes are also likely to be relevant in ASD pathogenesis, because their products are, next to neurotransmission processes, also involved in synapse maintenance and plasticity, and they play a pivotal role as neurohypophyseal hormone receptors in animal models of social interaction.

Second-messenger proteins such as neurobeachin (NBEA), an anchoring protein able to recruit protein kinase A, and protein kinase C β (PRKCB) I and II, are implicated in the differentiation of antigen-presenting dendritic cells whose dysfunction could contribute to altered immune responsiveness in ASD. These proteins are also involved in Ca^{2+} signaling, and perturbation of their synthesis could translate into altered synaptogenesis. Remarkably, while there is no putative genetic explanation, reduced programmed cell death leading to increased cell numbers and maintenance of misplaced cells are described consistently in neuropathological studies of ASD.

Secreted molecules, such as the reelin-protein (RELN), have a pivotal role in neuronal migration and prenatal development of neural connections. This has been confirmed in reeler-mice, which lack reelin and display cytoarchitectonic alterations in numerous brain regions. Post-mortem brain analysis of autistic individuals shows impaired reelin signaling and reduced blood reelin levels have been found in individuals with autism and first-degree relatives. RELN-variants that cause decreased reelin expression are suggested to confer vulnerability to ASD. However, the genetic heterogeneity and reported inconsistencies suggest interpretation of RELN-variants within a framework of a region-specific gene-

environment interaction model. For example, a gene-environment interaction is thought to occur between RELN-variants and exposure to organophosphates. The latter inhibit RELN-associated proteolytic activity on matrix proteins, which is essential for neuronal migration. This assumption is further strengthened by the finding that variants in the paraoxonase (PON) gene, which encodes the organophosphate deactivating enzyme paraoxonase, are linked with RELN-variants in ASD. The laminin β1 (LAMB1)-gene, encoding the β1-chain of laminin, is another interesting candidate. Laminin is an important glycoprotein promoting neuronal migration and neurite outgrowth in the developing nervous system. A knock-out mouse model of the engrailed 2 (EN2) gene (7q) lacking the EN2 homeobox transcription factor resulted in a hypoplastic cerebellum and decrease in the number of Purkinje cells. Neuropathology reminiscent to that of the EN2 knock-out mouse was observed in post-mortem brain studies of individuals with ASD. Moreover, reduced expression of EN2-gene products has been associated with PDD. Reduced expression of the MET gene has recently been implicated in autism [166]. MET receptor tyrosine kinase mediated signaling is involved in neocortical and cerebellar growth and maturation, immune function, and gastrointestinal repair, possibly explaining some of the comorbidity observed in ASD.

It can be concluded that individual genes have been implicated by means of their positional (through association or linkage) and/or functional (through their involvement in neurodevelopment and/or neurotransmission) properties. Findings of reduced programmed cell death and/or increased cell proliferation, altered cell migration with disrupted cortical and subcortical cytoarchitectonics, abnormal cell differentiation with reduced neuronal size, and altered synaptogenesis have been proposed to explain the unbalanced local versus long-distance and inhibitory versus excitatory connectivity possibly underlying altered social information processing in autism. The presented evidence implicates three pathways in ASD pathogenesis: (i) cell migration, (ii) glutamate-GABA equilibrium, (iii) synapse formation and maintenance, as well as dendritic morphology [146]. Genes that are implicated in these pathways encode proteins that can be divided according to their involvement in (i) chromatin remodeling (e.g. MECP2) and regulation of transcription (e.g. FMR1), (ii) actin cytoskeleton dynamics (e.g. TSC1, TSC2 and NF1), (iii) synaptic scaffolding (e.g. SHANK3), (iv) neurotransmission (e.g. SLC6A4), (v) second-messenger systems (e.g. NBEA), (vi) apoptosis, (vii) cell adhesion (NLGN), and (viii) paracrine cell-cell communication (e.g. RELN).

Recently, through the development of high-resolution genome analysis techniques to identify *de novo* genomic deletions and duplications of tens to thousands of kilobases (i.e. copy number variation; CNV), the number of cases with traceable

underlying genetic causes of autism has been raised to 10-20% [167;168] and this number may even grow to 30-40%. *De novo* CNV encompasses at least 12% of the human genome and hundreds of genes [169]. Copy number variation can result in the loss of copy, gain of copy and disruption of a dosage-sensitive gene all with effects at the protein level [170]. Copy number variation might contribute to the interindividual genetic variability even more than single-nucleotide polymorphism (SNP). The existence of a higher rate of CNV and also *de novo* CNV in ASD compared to controls suggests that genetic causes of autism have high heritability but mutations are not inherited, which is explained by the *de novo* aspect of CNV [171]. This new understanding explains a part of the puzzling data from twin studies in autism.

The potential existence of less penetrant CNV that has smaller effects but also contribute to autism is interesting. Copy number variation does not seem to increase with age in contrast to point mutations associated with increased paternal age. Beaudet [171] proposes a mixed epigenetic and genetic *de novo* and inherited model for autism (**Figure 2**), in which individual patients have a genetic (mutation) or epigenetic (epimutation) cause of autism and these components could be inherited in some cases and could be acquired *de novo* in others [172].

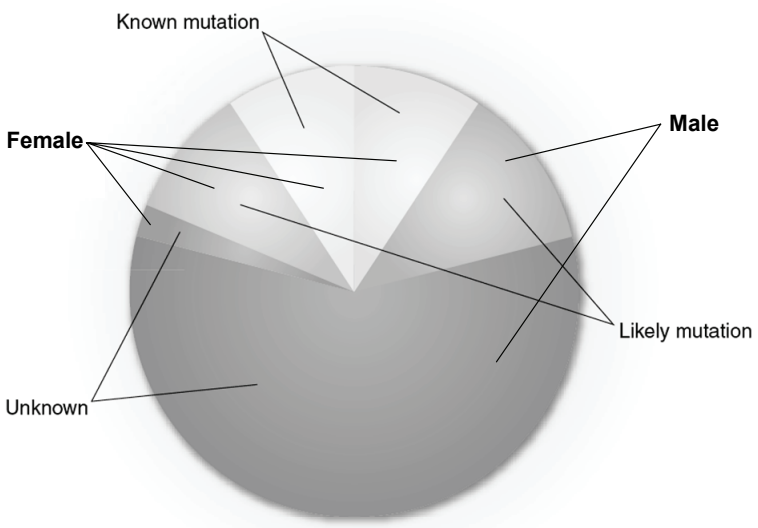


Figure 2.
From [171]. The causes of autism according to genetic contribution and sex.

In summary, results from (epi-)genetic and neuropathological studies in ASD and related behavioral and developmental disorders provide a putative role for genes involved in brain development and all aspects of neurotransmission that are determinants of behavior. These genes should be prioritized for future genetic research. Nevertheless, the impact of the environment should not be neglected as well as the modest effect sizes of individual genes and poor consistency of most implicated genes. Future studies should conform to guidelines for genetic analysis of complex disease [157].

4.3.1.4. Outlook and discussion

Additional research strategies for the identification of mental disorder susceptibility genes have been proposed: (i) use of mouse-models for study of gene-environment interactions, (ii) the study of genetics in a pedigree of a mental disorder accompanied by a specific somatic syndrome, (iii) study of mitochondrial DNA in cases where mental disorders are more frequently observed in maternal relatives of children with mitochondrial diseases or metabolic diseases compared with their paternal relatives, (iv) use of microarrays to study the expression of a large set of genes hypothesized or known to be involved in neurodevelopment, neuroplasticity, neurotransmission, and remodeling or migration of neurons. It is probably also beneficial to focus on single large families to avoid genetic heterogeneity, to take into account geographic origin, or to genotype isolated populations. Another important question is whether to use tissue, e.g. of the brain or other neuronal tissue, or lymphocytes for genetic analysis. The latter is thought to be less representative for gene expression in the brain [145]. However, genotype characteristics are inevitably related to psychiatric phenotypes classified by the DMS-IV and ICD10 criteria, spurring the subject of psychiatric nosology. Phenotype definition is generally difficult in mental disorders, because no sensitive and specific tests are available to distinguish different disease entities.

Another interesting notion is the significant publication bias, which has made the genetic research community to call for a platform for negative results. Interestingly, among the 166 putative associations with bipolar disorder studied at least three times, only six associations were replicated in more than 75% of the studies, which can be explained by small sample-size, population stratification, phenotype definition, genetic heterogeneity, low relative risk, multiple testing, genotyping error, selection bias especially for the control group, and many other factors [173]. Publication bias has likely compromised genetic meta-analyses and published positive findings may prove to be oversimplifications after more detailed analysis. For example, re-analysis of a polymorphic region of the 5-HTT-allele found it to be consisting of at least 14 alleles instead of the previously assumed 2

alleles. This finding is important, because different alleles have functional differences. Guidelines are in preparation to prevent the aforementioned problems and to increase the validity of future reported results.

Evenly important, the unequivocal identification of susceptibility genes caused by the advancement in genotyping raise ethical and psychological concerns regarding the availability of information and services for those under consideration for genetic testing. Age at testing, consent procedures, and post-testing implications for the individual require careful consideration of researchers and policy-makers. However, identification of causative genes provides an opportunity for genetic counseling, and via increased insight in disease mechanisms it may result in the development of effective therapies. For example, careful genetic assessment of children with ASD can be used to determine whether the autism is of syndromic or idiopathic nature. This will help to inform parents with regard to the recurrence risk (e.g. 2-8% for idiopathic autism) of the disorder and it will help them with the psychological coping with the impact of the disorder.

If future research discover genes and their specific pathogenic mutations that can be unambiguously linked to certain mental disorders or associated traits, the feasibility of the following increases: (i) rational drug design, (ii) characterization of genotype-phenotype relations, (iii) identification of environmental risk factors interacting with specific genes, (iv) realistic research into prevention focusing at identifying high-risk individuals [142], and (v) improved psychiatric nosology. It is important that future studies on the 'genes of mental disorder' account for epigenetic regulation of gene expression via interaction with the environment, because gene-mutations alone are not expected to explain the origin of the majority of mental disorders.

4.3.2. Epigenetics

Some evidence for the involvement of epigenetics in several mental disorders has already been presented in the previous section: altered epigenetic control of COMT, RELN, and glutamate decarboxylase (GAD_{67}) in schizophrenia and bipolar disorder, mutations in MECP2 and UBE3A in autism, and of the glucocorticoid receptor (GR) in anxiety and depression [174]. Interaction of our genome with the environment may be mediated through epigenetic modifications of our genotype. The epigenetically modified genome is referred to as the epigenome. Epigenetics refers to heritable, but reversible regulation of various genomic functions, mediated principally through changes in DNA methylation and chromatin structure [175] and non-coding RNA (ncRNA) [176]. The extent and consistency with which epigenetic modifications are transferred from one generation to another during

meiosis and from cell to cell during mitosis (for cell differentiation) are, however, still subject of debate.

A key issue of epigenetic modifications is their lasting effect on gene expression by either up- or down-regulation of gene expression. While the DNA sequence of an organism defines the primary structure of the proteins, epigenetic mechanisms control the quantity, location and timing of gene expression [55]. Epigenetic processes are thus essential for normal cellular development and differentiation, and are thought to be mitotically stable. Parent-of-origin specific effects, also referred to as genomic imprinting, are presumed to be under epigenetic control. Genomic imprinting is the differential expression of genetic material at either a chromosome or allelic level depending on whether the genetic material has been transmitted from the paternal or maternal side [55]. Two metastable epigenetic processes are transgenerational inheritance of phenotype (meiosis) and the interaction of the genome with the environment (fine tuning of phenotype).

Epigenetic control is exerted through cytosine methylation at CpG dinucleotides, the post-translational modification of histones by means of acetylation or methylation, ubiquitylation or small ubiquitin related modifier proteins-ylation (SUMOylation), phoshorylation and ADP-ribosylation, and transcriptional silencing and alterations of DNA-methylation by ncRNA [153]. CpG methylation occurs through DNA methyltransferase catalyzed transfer of a methyl-group (CH_3 or C_1 group) from S-adenosyl methionine (SAM) to cytosine residues. Histones may also be de-methylated or methylated by enzymes that abstract (e.g. demethylases) or transfer (e.g. methyltransferases) a methyl group. Other chromatin remodeling systems that have been implicated in epigenetic changes are nucleosome sliding (mediated by ATP-dependent chromatin remodeling proteins) and histone substitution (exchange of histones from nucleosome with external histones) [153].

Typically many genes show an inverse correlation between the degree of methylation and the level of expression [177]. Methylated CpG sites (2-5% of all DNA bases) are overrepresented in CpG islands of the promoter regulatory region of many genes. Methylation disrupts binding of transcription factors and attracts methyl-binding proteins that are associated with gene silencing and chromatin compaction. The X-linked gene encoding MECP2, e.g., is mutated in Rett's syndrome resulting in decreased MECP2 activity. Decreased MECP2 activity leads to transcriptional de-repression of specific promoters thereby decreasing the expression of genes encoding proteins which are crucial for brain development and plasticity [146]. There is a clear interaction between CpG methylation and histone modification. For example, methylated CpG islands normally recruit active MECP2, which in turn recruits co-repressors such as histone methyltransferase (HMT) and

histone deacetylase (HDAC) complexes. HDAC deacetylates histones thereby changing the chromatin structure from an activated, open state, which allows gene transcription (euchromatin), to an inactivated, condensed state, which does not allow gene transcription (heterochromatin). However, permissive and repressed intermediate chromatin states have also been suggested [153]. Chromatin remodeling has a high temporal and spatial resolution with regard to modulating gene expression by permitting or inhibiting access of the transcriptional machinery to specific promoter regions [153]. The diversity of histone modifications and their different spatial effects on chromatin structure enables the definition of a specific epigenetic state of gene activation or silencing also referred to as the "histone code hypothesis" [178].

Many researcher have adopted an epigenetic perspective in mental disorder research for the fact that it might explain a number of the observed phenomena [55;153]: (i) discordance of MZ twins, (ii) contribution of the environment, (iii) high heritability but slow progress in identifying risk genes, (iv) gene-environment interactions, (v) frequently observed unequal sex-ratios, (vi) parent-of-origin effects, (vii) gradual onset, chronic and remitting course over a lifetime, and (viii) necessity for chronic administration of psychiatric medication to mediate effects. Improved insight into these phenomena through the study of epigenetic processes will contribute to the development of new diagnostic methods and treatments.

Another important aspect of epigenetic traits is their potential heritability. It is not completely clear how epigenetic traits are transferred during gametogenesis and meiosis, or how they are maintained during mitosis. The answer to this question seems to be nuanced. In somatic cells the epigenetic profile is transferred from maternal to daughter chromatids during mitosis [55]. However, there seems to be considerable infidelity in the maintenance of methylation patterns in mammalian cells and *de novo* methylation events are fairly common during mitosis [179]. Especially unmethylated regions outside the promoter regions are unreliably inherited [180]. Metastability of the epigenetic profile during mitosis may have profound effects. Generally it is also assumed that epigenetic profiles are reset and erased during gametogenesis and early embryogenesis, but evidence is mounting that for at least some genes epigenetic marks (epi-alleles) are transmitted during meiosis and thus transmitted from generation to generation [181]. In addition, histone modifications, e.g. methylation, are potentially inherited through 'template reading' and 'writing' mechanisms or indirectly as a result of gene transcription. The coupling of DNA methylation replication and subsequent gene-silencing to modifications of newly-synthesized histones is of special interest and requires further investigation [182]. Taken together, the finding of only partial erasure of

epigenetic marks during gametogenesis has significant implications for heritability and inheritance research in mental disorders.

Because most neurons do not divide in the adult brain, chromatin modifications and DNA methylation are sustained within individual cells and affect activity, survival, and morphology of neurons and ultimately integrated regulation of complex behavior [153]. It is therefore that most epigenetic modifications associated with neurobiological adaptations are long-lasting. In the section dealing with genetics of schizophrenia, bipolar disorder, depression and PDD some epigenetic 'mutations' have already been linked to the increased risk or occurrence of traits/symptoms associated with these mental disorders. The following sections briefly discuss in more detail the possible role of epigenetics in the etiology and severity of these disorders.

4.3.2.1. Epigenetics of schizophrenia and bipolar disorder

The most widely studied epigenetic alteration in schizophrenia concerns that of genes aberrantly expressed in GABAergic neurons (e.g. RELN-promotor region methylation [183]), causing dysfunction of GABA-mediated neuronal circuitry. Recent studies conducted in post-mortem brains of patients with schizophrenia and bipolar disorder found increased expression of DNA-methyltransferase 1 (DNMT1), and reduced expression of RELN and GAD_{67} in GABAergic neurons in several brain regions of patients with schizophrenia [184;185] but not in patients with bipolar disorder [185] compared to non-psychiatric controls. Another study found 2-3 fold increased levels of SAM, the universal methyl-donor, in brains of patients with schizophrenia and bipolar disorder compared to patients with unipolar disorder and controls [186]. The increase in SAM levels in these patients was associated with DNMT1 overexpression in prefrontal cortex GABAeric neurons [186], which is suggestive for SAM-substrated and DNMT1-mediated RELN- and GAD_{67}-promotor hypermethylation and consecutive by reduced gene expression. S-adenosylmethionine is intimately linked to nutrition through C_1-metabolism [63].

Another candidate gene for epigenetic alteration is COMT. Recently, a 2-fold lower rate of promoter region methylation of the gene encoding for MB-COMT was found to be a major risk factor for schizophrenia and bipolar disorder [187]. The authors also found a corresponding increase in MB-COMT transcripts, an inverse relation with dopamine receptor D_1 (DRD1) and a tendency for the Val allele polymorphism to be associated with hypomethylation. This could cause degradation of dopamine at increased rates through overexpression of MB-COMT and hyperactivity of the COMT-allele together with secondary downregulation of DRD_1 expression. The latter is suggested to reduce RELN-gene expression through dopamine-DRD_1 interaction mediated cAMP response element hypermethylation

of the RELN-promoter region [187]. Treatment of patients with schizophrenia and bipolar disorder with epigenetic drugs such as inhibitors of methylating enzymes (e.g. DNMT1) might reverse promoter hypermethylation of suppressed genes. Noteworthy is the finding of DNMT1-inhibition by doxorubicin and subsequent reactivation of the human RELN and GAD_{67} genes in neuronal cell culture, genes that are down-regulated due to modifications in the epigenome [188]. Interestingly, the concentration of doxorubicin used for DNMT1-inhibition did not induce significant cell death, while it resulted in robust induction of RELN and GAD67 mRNA [188]. However, the cell, gene and promoter specificity and selectivity should be extensively investigated *in vitro* and in animal models before these potentially toxic compounds are tested in human subjects.

4.3.2.2. Epigenetics of depression

The persistence of depression is thought to be mediated by slow but stable adaptations, including those of epigenetic nature [153]. It is interesting that one of the proven therapies of depression, i.e. chronic electroconvulsive seizures (ECS) upregulates BDNF expression through changes in chromatin remodeling, which were distinct from changes in chromatin after acute ECS. BDNF upregulation mediates antidepressant activity in animal models [153]. The notion that altered epigenetic regulation is involved in depression is supported by the fact that chronic social defeat stress in an animal model of depression downregulates expression of BDNF splice variants, which is reversed upon chronic administration of imipramine [189]. The possibility to use specific HDAC, HMT and histone demethylase (HDM) inhibitors to treat depression is raised by some [153].

Interestingly, in rats, offspring of mothers with high licking, grooming and arched-back nursing (LG-ABN), display increased expression of glucocorticoid receptor mRNA and protein, and decreased GR promoter methylation compared to pups of mothers with low LG-ABN (reviewed in [190]). The difference in promoter methylation emerged in the first week of life, persisted into adulthood but could surprisingly be reversed by cross-fostering. Furthermore, pups with high LG-ABN mothers have increased expression of nerve growth factor inducible factor A (NGFI-A), which binds the GR promoter regions and enhances its transcription [191]. The third argument in favor of a role for epigenetic contribution to the etiology of depression comes from proband sex effects.

Skewed X-chromosome inactivation is an epigenetic process which might explain the excess rate of unipolar depression in women and the female MZ twin discordance [192]. It is generally assumed that in women X inactivation is stochastic for each cell lineage and that this is maintained throughout subsequent cell divisions. However, in 5-20% of women without X-linked disorders, there is

constitutional skewing of X-inactivation [193]. Moreover, it seems that as many as 15% of genes are expressed from both X-chromosomes [194]. These genes are obvious candidates for explaining sexual dimorphism in disease prevalence in women [55]. Another explanation for sex effects of depression might be mediated by hormone-specific modification of certain genes. A fourth argument is a parent-of-origin effect, i.e. disease susceptibility is mediated by parental factors in a sex-specific manner, e.g. preferential maternal transmission of a GluR7 gene risk allele to patients with recurrent depression has been reported [195]. Genomic imprinting seems to be the underlying mechanism. Last, the interaction between the genotype and epigenotype may be commonplace, and it may better predict the risk of developing mental disorders such as depression. For example, the T-allele of the C677T polymorphism in the MTHFR gene is implicated in depression [196] and the Val allele of COMT has been associated with promoter hypomethylation [187]. These results suggest it is better to perform comprehensive analyses of both the genotype and epigenotype to evaluate whether some epigenetic changes may be associated with specific polymorphisms. Especially for complex diseases with a large environmental component it is unacceptable to study the contribution of genes without the study of DNA methylation and histone modification.

4.3.2.3. Epigenetics of autism

The causative role of epigenetic mechanisms in autistic-like behavior is best exemplified in fragile-X syndrome in which CGG repeat expansion in the FMR1 gene renders it susceptible for epigenetic silencing and subsequent reduced FMR1 expression, and in Rett's disorder heterozygous females in which the gene encoding a key-modulator in epigenetic control (MECP2) is mutated [152]. MECP2 brings about silencing by binding to methylated promoters and recruiting co-repressors and histone-deacetylases. This alteration of gene-expression by decreased MECP2 activity leads to altered levels of products of the following genes: BDNF ↑, distal-less homeobox 5 transcription factor (DLX5) ↑, UBE3A ↓ and GABR β3 ↓ (reviewed in [146]). Expression of the RELN-gene is modulated by promoter methylation [124]. In addition to altered epigenetic control of single-gene expression, parent-of-origin effects and genomic imprinting have been implicated in PDD [197]. Especially in Angelman syndrome and PWS the effect of genomic imprinting is clear (see 'genetics' sections). The aforementioned gene products are implicated in brain development and plasticity. Next to other chromosomal regions subject to imprinting, imprinting errors of 7q and 15q have been associated with Angelman syndrome, PWS and Turner syndrome (X monosomy) [146;152]. The gender bias in autism may also have epigenetic roots, because it is maintained after exclusion of syndromic ASD cases with X-linked genetic origins. An epistatic mechanism in

which genes involved in neurodevelopment and neural signaling interact with genes encoding for products having endocrine functions has been suggested, but could not be confirmed. One suggestion is that maternally derived X-linked loci are silenced and thus not expressed in males, rendering them more vulnerable to impairments in social and communication skills [198].

Taken together, epigenetic mechanisms definitely play an important role in the etiology of mental disorders and it is thus of the utmost importance to take these effects into account when performing analyses directed at the 'genetic' origin of such disorders. With this in mind the function of ligands for transcription factors, e.g. certain long-chain polyunsaturated fatty acids (LCPUFA), and methyl-donors, e.g. folate, requires closer examination because these compounds explain many risk-factors, and gene-environment and environment-gene interactions that are associated with the development of schizophrenia and autism [63]. Furthermore, it is important to recognize the very exciting possibility that we inherit next to the DNA of our ancestors also their lifestyle. It is most likely that the purported genetic and epigenetic changes are observed as differential expression of proteins and metabolites in tissues and bodyfluids. Profiling of protein expression and metabolite abundance could shed more light on the consequences of these changes.

5. Biomarkers for mental disorders

In the previous paragraphs we have dealt with the socioeconomic burden, epidemiology, diagnosis, classification and the etiology of mental disorders. With regard to the vast amount of research that has been performed to mental disorders it is fair to question what knowledge is still needed with regard to prevention (ranging from pre-pregnancy measures to risk-factor reduction in adolescence), diagnosis (psychiatric nosology based on definitive objective and preferably quantifiable measures with a link to etiology) and treatment (effective treatment adapted to an individual's geno- and phenotype) as well as outcome monitoring (remission and relapse estimation) of mental disorders. The review of literature describing hypothesis-driven research may seem somewhat disappointing with respect to findings of generally applicable biomarkers for these purposes. For example, still no diagnostic blood test is available for any mental disorder and treatment outcome monitoring is usually done by assessing a patient's behavior and socioeconomic functioning. Genomic, transcriptomic, proteomic and metabolomic approaches using an in-depth qualitative and quantitative assessment by expression profiling of the respective '-omes' in health, the target disease and closely related diseases, may generate biomarkers that comply with criteria for diagnostic or therapeutic markers.

A consensus definition of a biomarker, shorthand for biological marker, is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [199]. Another definition of a biomarker is "a measurable indicator of a specific biological state, particularly one relevant to the risk of contraction, the presence or stage of disease" [200]. Nowadays, the term 'biomarker' is often used to refer to a molecular biomarker but physical traits or physiological metrics (e.g. results from molecular imaging) should be considered [200].

Biomarkers can be used for the diagnosis, staging, screening and prediction of a disease and for monitoring the effectiveness of and patient compliance with a treatment [201;202]. With respect to mental disorders, there is a great need for biomarkers that enable the development of objective, differential diagnostic systems that enable treatment monitoring. The identification of distinct phenotypical subtypes through the use of biomarkers may complement and even replace current diagnostic criteria. The combined use of biomarkers and endophenytopes for early recognition [203] and classification may be another promising approach. Moreover, the identification of biomarkers and other endophenotypes would likely facilitate the setting up of effective programs for prevention and early detection of mental disorders leading to better treatment outcome and a less severe course of the disorder.

Biomarker research into the causes, traits, states and treatment options of patients with mental disorders should be stimulated by governmental institutions for matters of cost-effectiveness. In addition, the psychiatric community is likely to benefit from an objective measure for the classification and treatment of patients with mental disorders. To achieve this, a multidisciplinary approach and multilevel analysis of the results is essential. Unfortunately, using hypothesis-driven research no single biomarker or panel of biomarkers has been discovered that fulfils the criteria for a diagnostic or surrogate-end-point marker. Other approaches complementing hypothesis-driven research are therefore being pursued integrating results from genomic, transcriptomic, proteomic and metabolomic studies.

5.1. Diagnostic biomarkers for mental disorders

A diagnostic biomarker test for a mental disorder preferably should fulfill the following eight criteria [204]: (i) detect a fundamental feature of disease with high sensitivity and specificity, (ii) be validated in confirmed post-mortem cases, (iii) be standardized with proper bioinformatics and proper statistics, (iv) be specific for the disease compared with related disorders, (v) be reliable in many testing environments/labs, (vi) be preferably non-invasive, (vii) be simple to perform, and (viii) be inexpensive. Early diagnosis of schizophrenia, for example, is important

because a longer duration of untreated psychosis after the first manifestation is associated with a deteriorating prognosis. Early intervention, already in the prodromal phase, in these patients reduces the period of untreated psychosis and sometimes it even prevents or delays the onset of psychosis in high-risk individuals [205]. In addition, early intervention improves social and functional outcomes and is cheaper than standard care models that start treatment once a psychosis is manifest [205]. Early intervention in children with autism is likewise associated with a better prognosis [49]. The current diagnostic systems, i.e. DSM-IV and ICD-10, are neutral with respect to theories of etiology. These systems therefore only provide limited insight into which therapy, either pharmacological or psychosocial, is most effective.

Unfortunately, a number of factors hampers biomarker discovery in mental disorders: the absence of an objective biological 'gold standard', frequently prevalent psychiatric comorbidity, heterogeneity and equifinality (same symptomatic or syndromatic clinical diagnostic entity represents different initial conditions that lead to the same clinical endpoint), quantitative traits or intermediate phenotypes and a presumed polygenic/multifactorial etiology [206]. It is also virtually impossible to study the primary affected organ in humans, except for special cases, where cerebrospinal fluid may be taken. Novel approaches of molecular imaging may also allow a more detailed study of brain disorders in humans. A generally applicable biochemical diagnostic marker should, however, be preferably detectable and quantifiable in either peripheral cells, tissues, or body fluids that are easily accessible to be routinely examined in patients. Another major problem is to determine the proper cut-off level of a biological marker or a marker panel: where does normal interindividual variability end and where does pathology start [207]?

Functional tests, e.g. the dexamethasone suppression test (measures the cortisol- response of the adrenal glands to ACTH) that is used to study the HPA-axis, have also been proposed as useful tools in the diagnosis of mood disorders. Despite the poor sensitivity and specificity of the dexamethasone suppression test for patients with depression in general [206], it is a quite well-established predictor of risk of suicide in depressed patients, along with serum cholesterol [208]. If future research uncovers other pathophysiological mechanisms or affected systems that are specific to certain mental disorders, functional tests may be devised that address hypo- or hyperactivity of these systems. Functional testing may provide an alternative path that also provides valuable information with respect to the proper diagnosis and to the most effective treatment.

5.2. Biomarkers for treatment monitoring of mental disorders

Biomarkers for monitoring treatment and outcome for mental disorders are less easily qualified than those for somatic disorder like diabetes. In the (bio)-pharmaceutical industry biomarkers are qualified according to whether they are "fit-for-purpose" [209]. Exploratory biomarkers are used as research and development tools accompanied by some preliminary clinical evidence. Their main purpose is to support the generation of new hypotheses (e.g. gene expression profiling). Valid exploratory biomarkers have demonstrated, but not yet reproducibly, adequate preclinical sensitivity and specificity and are linked to clinical outcome. These biomarker candidates can assist in clinical decision-making (e.g. adiponectin). Fully validated biomarkers have reproducibly proven their adequacy in terms of sensitivity, specificity and their link to clinical outcomes in multiple prospective clinical studies in human. These biomarkers are also used for dose finding in clinical trials and secondary/tertiary claims (e.g. fasting plasma glucose). If a biomarker can function as a substitute for a clinical endpoint it is designated as a surrogate end point. These biomarkers can be used for registration purposes of new pharmaceuticals (e.g. hemoglobin A1c). However, few biomarkers make it from the exploratory phase to the final status of surrogate end point.

A biomarker that may serve as a surrogate end point is most interesting in psychiatry, since clinical endpoints of mental disorders are diverse and not well-defined. The most important indicators of recovery from a mental disorder are: having paid work fit to the patient's educational background, having normal interpersonal relationships and being devoid of any symptoms that impair daily living. Of course, it will be very hard to link these complex psycho-socioeconomic outcomes to any biochemical marker. It thus seems more feasible at present to use biomarkers for the identification of novel pharmaceutical targets and compounds that share the same mechanism of action. Biomarkers may also be valuable tools to validate animal models for mental disorders and to translate preclinical results into the clinic. The study of efficacy and toxicity of new drugs in animal models with predictive power for human clinical trials is another field of application [204].

5.3. Biomarker discovery

5.3.1. Tissue banks and sample collections

Hypothesis-driven biomarker research in mental disorders focuses at those tissues, organs, cells and pathways which are implicated in the etiology and severity of the disease, because of their obvious relation with symptoms. Though this approach has yielded reasonably effective treatments for some mental disorders, it may overlook more hidden biomarkers because of spatial and temporal constraints. For example,

peripheral cells are easier to obtain than post-mortem brain tissue and a spatial constraint might thus be that the investigation of peripheral tissue or cells (e.g. leukocytes) may not fully resemble molecular and cellular processes in the brain, although this is disputed by some [210]. Analysis of fluid more proximate to the affected organ than blood or urine, such as CSF, is preferable but also more difficult to obtain without a clear medical indication. With respect to the temporal constraint, one may have to conclude that earlier and current research has exposed only those abnormalities secondary to the primary causative process. The presumed neurodevelopmental origin of many mental disorders suggests that biomarker studies should start before clear symptoms of the disease are visible. Disease entities with similar symptomatology but different underlying causes can probably not be differentiated by the study of secondary processes. All together, it may be that the search for true diagnostic markers that can differentiate between psychiatric disorders with different etiologies was unsuccessful because we were just too late and studying the wrong thing.

To circumvent these problems large-scale biobanking projects have been set up that coordinate the collection of whole-blood, serum, plasma, erythrocytes, leukocytes (for DNA) and other biofluids (e.g. urine, CSF) and/or post-mortem brain tissue from affected individuals and controls. For proper biobanking, it is also necessary to collect samples from affected and unaffected relatives. Especially a collection of biofluids and pregnancy tissues from the mother before and during pregnancy of the affected individual is important to define vulnerability windows of intrauterine neurodevelopment and their association with psychiatric disorders in later life. For the father pre-pregnancy DNA might be considered important because of changes in epigenetic markers over time and imprinting effects.

Several post-mortem brain collections (http://www.intbbn.org/) are available for studies of schizophrenia, bipolar disorder and depression: the Stanley Foundation brain collection [211], the Oxford brain bank [212], the Harvard brain collection [213], the BrainNet Europe Consortium [214] and others. The tissue in these banks is generally accompanied by the necessary information. Generally, brain tissue is dissected into several brain regions that are sliced and stored as freezing coupes. These brain slices give an opportunity to study different affected regions resulting in increased homogeneity of the sample. Collaboration between tissue banks and standardized collection of tissues as well as extensive accompanying information is the key to successful exploitation of these banks, because only then will sample size be sufficient to control confounding factors and to draw firm conclusions. For autism research, the Brain and Tissue Bank of the University of Maryland is probably the best established collection [215]. Several other initiatives, notably in the USA, have led to the collaboration of different brain

tissue banks to increase the number of samples for the study of (neuro-) developmental disorders. This is necessary because of the low prevalence and decreased likelihood of early death in children with PDD.

Several population cohorts such as the Northern Finland 1966 Birth Cohort Study, the Danish population-based cohort studies, and the NIMH (National Institute of Mental Health) epidemiological catchment area studies [216] have increased the understanding of mental disorders significantly. Prospective collection of blood specimen, DNA together with epidemiological information of both affected individuals, their (un)affected relatives and controls is to this moment less common practice. If biobanking is to be implemented in these cohort studies, standardization of sample collection, sample storage and sample management is of utmost importance. An example of DNA-biobanking in PDD is the International Molecular Genetics Study of Autism Consortium (IMGSAC) initiative from 1994. IMGSAC has provided many researchers with genetic material and clear diagnostic and anthropometric information of a sufficient number of patients to test their hypotheses. These and other large-scale initiatives provide the international research community with a wealth of high-quality samples and information. New diagnostic and therapeutic biomarkers for mental disorders are likely to come from the analysis of these tissue banks (e.g. the human proteome organization (HUPO) brain proteome project (BPP; http://www.hbpp.org/)) using non-hypothesis driven techniques. These techniques assess in a qualitative, semi-quantitative and/or quantitative fashion DNA (genomics), RNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics). The complementary and integrated analysis of these '-omics' data allows evaluation of complete systems using a systems biology approach [217].

5.3.2. Multi-analyte analysis by '-omics' technologies in biomarker research

As mentioned before, many '-omics' techniques use multi-analyte profiling and subsequent analysis of data using bioinformatics to extract useful information from the large amount of data that are generated by these techniques. Generally this causes the problem that datasets contain a low numbers of samples (10's-100's) and a large numbers of variables (1000's-10000's). This is also referred to as the high-dimensionality small-sample-size (HDSS) problem [218]. The analysis of properly collected and well-characterized samples by sophisticated low-throughput '-omics' techniques and subsequent data analysis by appropriate bioinformatics approaches that control or correct for the HDSS problem, enables the extraction of valuable information from which a diagnostic or therapeutic biomarker (or set of biomarkers) may be derived. Unfortunately the rate of introduction of new

diagnostics coming from these '-omics' techniques has been disappointing until now.

While large investments are being made in this area, the rate of introduction in the clinic of new protein biomarkers is falling, at least for cancer diagnostics [219]. The regulatory part of this problem is tackled by the creation of a consortium consisting of regulatory institutions (e.g. food and drug administration; FDA) and (bio)-pharmaceutical industries. This consortium focuses at the evaluation of cost-effectiveness and the qualification of biomarkers for regulatory decision-making [220]. In addition, researchers have begun to propose coherent and comprehensive processes (pipelines) for biomarker development consisting of: discovery, qualification, verification, research assay optimization, clinical validation and commercialization [200] in accordance to guidelines defining the minimal information about experiments for genomics and transcriptomics (MIAME), proteomics (MIAPE) and metabolomics (MIAMET). These pipelines are designed to provide some directions in facing the challenges associated with biomarker discovery. For proteins in blood, these challenges are related to the complexity and dynamic range of proteins in biofluids, the low relative abundance of many disease-specific biomarkers and the extent of variation between individuals and within a given disease [200].

Genomic and transcriptomic methods profile genes or gene-expression using microarrays of PCR-amplified material. However, gene expression data do not consistently correlate with protein expression and cannot identify post-transcriptional (e.g. alternative splicing) or post-translational modifications, which are major modulators of protein function, and presumably pathogenesis [217]. Proteomics and metabolomics may better reflect the dynamics of states and traits of patients. For proteome and metabolome profiling highly selective, sensitive and in certain cases also specific assays are required and these assays are usually preceded by extensive pre- and/or sub-fractionation methods (restricted-access chromatography, affinity-chromatography, depletion of high-abundant proteins etc.). Compared to the enormous number of articles about cancer proteomics, the input of proteomics and metabolomics in psychiatry research is scarce [204]. Some proteomic and metabolomic approaches have been applied to schizophrenia (proteomics reviewed in [204], [221-227]; metabolomics: [222;228]), bipolar disorder [proteomics [221;229]], depression [proteomics [221;223;230]; metabolomics [231]] and autism [proteomics [232;233]], or related animal models [all proteomics: schizophrenia [234;235]; depression [236;237]; anxiety [238]]. However, most of these studies have only shown changes in high abundant markers that are less likely to be related to the primary disease process. As a consequence

these studies have made limited contributions to the understanding of these disorders.

Routinely applied proteomics techniques are two-dimensional gel electrophoresis for protein analysis (2-DE) or liquid chromatography hyphenated with mass spectrometry (LC-MS). These techniques and their application in proteomics have been extensively reviewed [239-241]. In this review no further attempts will be made to review these techniques with respect to their advantages and drawbacks for biomarker discovery. We will merely describe the workflow (**Figure 3**) and challenges that proteomics faces in finding biomarkers for mental disorders as well as the findings of proteomic and metabolomic studies in this area.

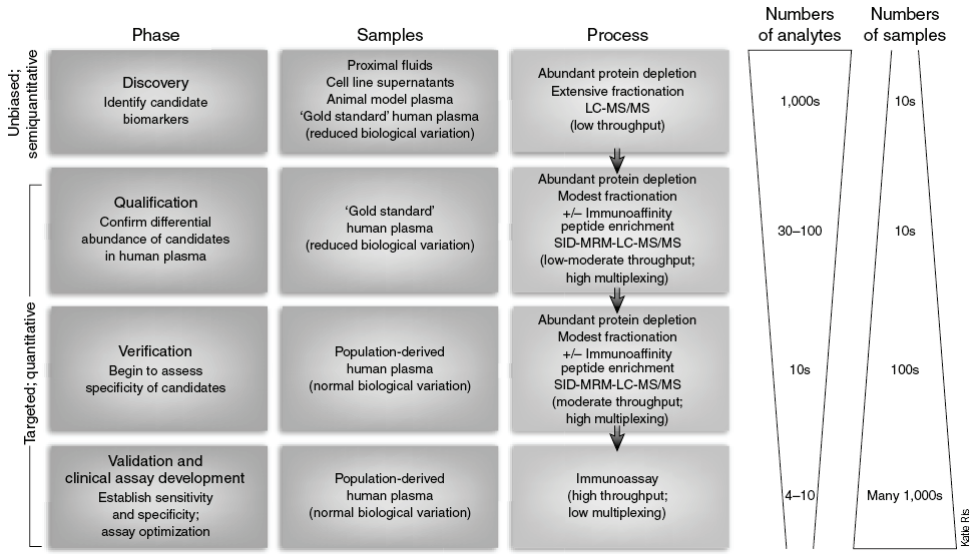


Figure 3. ***Process flow for the development of novel protein biomarker candidates***
From [200]. 'Numbers of analytes' refers to the number of proteins expected to be evaluated as candidate biomarkers in each phase of development. 'Numbers of samples' refers to the sample requirements for each phase. LC-MS/MS, liquid chromatography tandem mass spectrometry; SID, stable isotope dilution; MRM, multiple reaction monitoring.

5.3.3. Biomarker discovery pipeline in mental disorders and challenges

Biomarker discovery starts with the proper selection of patients and controls. Including large groups of patients having different but related mental disorders (symptom domains) according to the DSM-IV or ICD-10 criteria and a control group seems the best approach to find a diagnostic marker or marker panel. However, for the analysis of data an unbiased/unsupervised approach should be

used, since DSM-IV and ICD-10 classifications are not based on etiology. Furthermore, proper matching for age, gender, medication, duration of illness, lifestyle and other factors is of imminent importance to reduce the effect of confounding factors. For '-omics' studies of animal models for psychiatric disorders similar considerations are valid.

The next step in biomarker discovery is the selection of relevant samples, which can be biofluids such as blood or its derivatives (serum, plasma or specific cells), urine, CSF, or tissues (e.g. specific areas of post-mortem brains). Biofluids and their protein constituents are generally used in diagnostics and are thus a logical starting point for biomarker discovery, because of their comprehensiveness and accessibility [200]. Unfortunately, blood contains tens of thousands of proteins, which span ten to eleven orders of magnitude in concentration [200]. Disease-specific markers coming from the brain are likely to be highly diluted in blood and even more diluted in urine, which makes their discovery challenging. Higher concentrations of these markers are more likely to be found proximally to the affected brain in the CSF. A disadvantage of CSF is that it is not readily available, because it can only be acquired by an invasive lumbar puncture in limited volume (mL-scale). Analysis of post-mortem brain tissue is interesting, because it provides direct access to the affected organ, thereby increasing the chance of finding abnormalities that relate directly to the symptomatology. However, post-mortem brain analysis often means analyzing samples of convenience with little control over matching and confounding factors. In addition, brain abnormalities in later life might not be reflective of early processes that are responsible for the onset of, for example, depression or psychosis. Independent of the nature of the sample, samples should be collected in a standardized fashion and should be accompanied by the relevant information in a way that global exchange of material is possible.

Sample pre-treatment and analysis is the next step in the workflow. Often, sample pretreatment is crucial to reach into the lower concentration range of proteins and metabolites. For this, immunodepletion to remove high abundant proteins and affinity-chromatography to enrich proteins with specific post-translational modifications (e.g. glycosylated proteins) or a given functionality are frequently exploited [242]. Another interesting approach is the use of the blood peptidome, which accumulates on high-abundant circulating blood proteins like albumin, thereby serving as concentrators of potential disease-specific peptide markers [243].

Sample analysis is done using relatively low-throughput techniques, because extensive prefractionation must be generally performed. Consequently, one sample results in multiple fractions that need to be analyzed, for example, by LC-MS [242]. This improves sensitivity when hyphenated with hybrid mass spectrometers

exhibiting high mass resolution and mass accuracy, (e.g. linear ion trap/Fourier transform ion cyclotron resonance (LTQ/FT) or LTQ/Orbitrap mass spectrometers [200]). However, such an approach results in many tens of hours analysis time per sample [218]. Each sample may generate data files on the order of hundreds to thousands of Mbytes. There is thus an increasing need for data storage, processing and analysis capacity that should be managed by skilled bioinformaticians with an understanding of analytical chemistry.

Analysis of high-dimensional data from relatively few samples requires extensive knowledge about multivariate statistics and methods to reduce data complexity (binning/meshing), to extract relevant information (noise-reduction/peak-detection [244]) and to render LC-MS data comparable (normalization [245] and alignment of data in m/z and/or time domains [246]). Data processing methods that reduce data complexity by removing redundancy caused by isotopes and charge-states in the raw data have to be integrated in the data-processing workflows [247]. After data processing a matrix is constructed that contains values (intensity/area/volume) of individual peaks (for example, characterized by retention time and mass-to-charge ratio) from different samples (organized in columns). This matrix is subjected to unsupervised or supervised multivariate statistical analysis and visualization [218].

Depending on the goal of the study, multivariate analysis is performed in an unsupervised or supervised manner. In the search for a diagnostic biomarker or biomarker panel that differentiates between bipolar disorder and schizophrenia it is advisable not to use supervised classifying algorithms, because an intermediate phenotype might exist that affects sample classification and that would go undetected by using a supervised classification algorithm. In those cases it is better to use unsupervised principal component analysis (PCA) for class discovery, which in this example might result in the discovery of three classes (e.g. bipolar disorder, intermediate phenotype and schizophrenia) instead of the expected two. On the other hand unsupervised PCA reduces the chance of finding significant differences between classes, which makes it a risky strategy and at the end of the line it may prove an unviable option. The separation of groups of samples can be visualized by the projection of individual samples in a 2D- or 3D-plot consisting of principal components (PC) 1, PC 2 and PC 3. In contrast to diagnostic biomarkers that differentiate between psychiatric disorders, treatment biomarkers and biomarkers distinguishing between controls and affected individuals are more likely to be discovered through the use of supervised classification algorithms. Supervised classification pinpoints to those compounds that best discriminate a group of individuals or individuals before and after treatment. Next to the multivariate analysis of cross-sectional data, multivariate correlation analysis of longitudinal data

of groups of individuals is probably even more sensitive in finding biomarkers, because intra-individual noise is better estimated and corrected for.

The output of the multivariate analysis consists of a number of peaks representing one or more parent compounds. To decide which of the discriminatory peaks should be further studied (for example, after identification by LC-MS/MS), visual inspection, univariate statistical analysis and confirmation in an independent dataset should be performed. Confirmation of the discriminatory properties of identified compounds can be accomplished through spiking experiments or through dedicated analysis schemes (e.g. immunoassays; selective LC-MS/MS approaches).

The last step in the biomarker discovery workflow is the interpretation of the role of the discovered biomarker(s) in disease etiology and its validation in larger groups of patients in comparison with controls. Systems biology may be helpful in relating a given biomarker to disease etiology [217] as may be the study of the literature. Qualification, verification and validation according to FDA-guidelines and the Clinical Laboratory Improvement Amendments law of 1988 guidelines of the biomarker in larger groups should be performed with high-throughput assays such as stable-isotope dilution (SID) multiple-reaction-monitoring (MRM) LC-MS/MS on a triple quadrupole mass spectrometer or with sensitive immunotechniques such as radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA) [200]. For clinical validation is important to consider the consequences of the chosen (multivariate) cut-off(s). At least sensitivity, specificity and preferably also the negative and positive predictive value should be assessed and compared with other existing assays through receiver operating characteristics curves (ROC). If the marker holds with respect to clinical and analytical validation, it can be implemented in routine care.

5.3.4. Results from proteomic and metabolomic studies in mental disorders

Compared to cancer research, few studies report the use of '-omics' technologies in psychiatry research. In this section we focus on studies that used general proteomics techniques such as 2-DE, LC-MS and MS for the simultaneous analysis of proteins in brain tissue, CSF, plasma and serum as well as on some metabolomics studies that have used special analytical platforms usually GC-FID of derivatizated analytes or ^{1}H-NMR for profiling of glucose, fatty acid and oxidative phosphorylation pathways etc.

Advances of proteomics technologies in schizophrenia research have recently been reviewed [204]. Using 2-DE of the hippocampal proteome of seven patients with schizophrenia and seven controls, 108 differentially expressed proteins were

found. Among these were the diazepam binding inhibitor (DBI) and manganese superoxide dismutase (MnSOD) (both decreased), and the overexpressed collapsing response protein 2 (CRMP-2) and t-complex protein 1 (TCP-1) [248]. The first two proteins are involved in the regulation of GABA-ergic activity and in antioxidant activity, respectively [204]. TCP-1 aides in protein folding and arrangement. CRMP-2 is involved in axon formation. An alteration in turnover of the cytoskeleton through differential post-translational oxidation/nitration was suggested to be ongoing in the brains of patients with schizophrenia [248].

Another study of 89 frontal cortices obtained post-mortem from individuals with schizophrenia (n=24), bipolar disorder (n=23), major psychiatric disorder (n=19) and non-psychiatric controls (n=23) used 2-DE and MS sequencing of proteins [221]. Eight differentially expressed proteins were found: glial fibrillary acidic protein and dihydropyriminidase-related protein 2 were decreased in psychiatric patients, while ubiquinone cytochrome c reductase core protein 1 was only decreased in depression. The authors concluded that because some alterations in proteins were found in patients with different psychiatric diagnoses they may represent features that are common to the different diseases such as non-specific markers of inflammation or a common second messenger pathway [221].

Analysis by LC-MS of trypsin-digested serum proteins from 69 children with autism and 35 typically developing children showed only few differentially expressed peptides that barely reached statistical significance [233]. These peptides could be related to relatively high-abundant proteins such as apo-B100, complement factor H related protein, complement C1q and fibronectin 1, which do not seem to be very specific for autism and may reflect alterations in lipid metabolism and/or the immune system. 2-DE post-mortem brain tissue analysis of individuals with autism revealed a more acidic (polar) form of glyoxoalase I (Glo1) after identification of differentially expressed protein by LC-MS/MS [232]. This more acidic from of Glo1 was traced to a SNP in the Glo1 gene. The gene-product of Glo1 gene was found to have decreased enzyme activity and was later termed as predisposing factor in the etiology of autism [232].

Analysis of CSF from ten patients with schizophrenia and ten controls using 2-DE and MS protein sequencing showed downregulation of apolipoprotein A-IV (apo-AIV) in schizophrenia [249]. Decreased expression of apo-AIV was suggested to lead to reduced satiety signaling thereby increasing the risk of weight gain and insulin-resistance in patients using atypical antipsychotics [204]. MS sequencing of a differentially expressed protein in 2-DE analysis of plasma from treatment-resistant patients with schizophrenia and chlorpromazine-treated rats showed a decrease of plasma apo-AI in patients with schizophrenia and an increase in chlorpromazine-treated rats [227]. This suggests an association of decreased apo-AI

with the pathology of schizophrenia and an association of increased apo-AI with the therapeutic action of chlorpromazine [227]. The 2-DE analysis of CSF from patients with major depression that aimed at finding differences in the CSF-proteome between suicide attempters and non-attempters is another example of how proteomics can be applied to a clinical question [230].

Another frequently used proteomic technique is surface-enhanced laser desorption/ionization (SELDI) time-of-flight (TOF) MS. This technique was applied for the analysis of post-mortem dorsolateral prefrontal cortex from 34 individuals with schizophrenia and 35 controls [250]. Using a combination of 10 peaks the authors were able to diagnose schizophrenia patients with a sensitivity and specificity of around 70% after cross-validation. However, none of the peaks was identified, diagnostic performance was poor and confounding by medication was not controlled for, raising questions about the applicability and usefulness of this SELDI method and study design. SELDI-TOF analysis of CSF from patients with drug-naïve first-onset schizophrenia, depression, OCD and Alzheimer was more successful with a sensitivity of 88% and a specificity of 95% of the discriminatory proteins in an independent test-set [223]. Up-regulation of a VGF-derived peptide and down-regulation of transthyretin, which was co-regulated with a peptide cluster, were found to be discriminatory in this study [223]. Although CSF is generally not collected for the diagnosis of schizophrenia, a CSF-based diagnostic assay seems feasible, and it is of interest to see whether such an assay can also be used for treatment monitoring. Recently, SELDI-TOF MS protein profiling of dorsolateral prefrontal cortex tissue of patients with schizophrenia and bipolar disorder in comparison with controls revealed different groups of proteins involved in cell metabolism, signaling cascades, regulation of gene transcription, protein and RNA chaperoning, and other aspects of cellular homeostasis that could differentiate between the diagnostic clusters or between psychiatric patients and controls by their up- or downregulation [229]. Protein identification was done using matrix-assisted laser desorption-ionization TOF post-source decay MS [229]. Integrated transcriptomic, proteomic and metabolomic analyses of post-mortem prefrontal cortex from 10 patients with schizophrenia and ten matched controls suggested altered mitochondrial energy metabolism and oxidative stress in schizophrenia (in 9/10 patients) [222]. These findings were suggestive for low-grade hypoxia and energy depletion in the prefrontal cortex caused by reduced cerebral blood flow [222]. This study is a fine example of how systems biology can improve the understanding of disease mechanisms and at the same time provides new directions for research.

To improve insight into disease mechanisms and metabolic side-effects of three atypical antipsychotics, a metabolomics platform capable of quantifying 300 lipids

was used to analyze plasma of patients with schizophrenia before and after treatment [228]. Olanzapine and risperidone seemed to affect a broader range of lipid classes than aripiprazole and the former two caused increases of triacylglycerols and decreases of free fatty acid levels in contrast to aripiprazole, which may be related to metabolic side-effects. Interestingly, phosphatidylethanolamine (PE) levels increased after treatment for all drugs, suggesting PE-levels to be associated with therapeutic benefit. Moreover, metabolome profiling may be a promising tool for the identification of therapeutic response markers and non-responders (i.e. pharmacometabolomics), and to assess metabolic side-effects [228]. A metabolomics approach has also been adopted to advance knowledge about late-life depression [231]. Using GC-MS, plasma from depressed, remitted and never-depressed adults was studied. Remitted patients resembled never-depressed adults with respect to their metabolome, and depressed patients had altered levels of several fatty acids, glycerol and GABA, suggesting altered lipid and neurotransmitter metabolism.

The application of proteomics and metabolomics in animal models of mental disorders has been diverse: (i) validation of animal models by studying the underlying pathology (e.g. mechanism by which methamphetamines bring about behavioral sensitization [226]); (ii) investigation of changes caused by the NMDA-receptor antagonist MK-801 (compound with psychotomimetic effects) in the thalamus [235] and cortex [234]; (iii) discovery of anxiety-related proteins in mouse brains [238]; (iv) observation that enduring high levels of circulating cortisol lead to altered cellular morphology and cell death pathways [237]; (v) for monitoring of the effects of medication. The effects of chlorpromazine [225;227] and clozapine [225] treatment on the plasma proteome in rats were studied and a study to the effects of monoamine reuptake inhibitors such as fluoxetine and venlafaxine showed changes within the hippocampal formation, beyond 5-HT/norepinephrenine neurotransmission, which may reflect long-term functional adaptations that are required for antidepressant activity [236].

5.4. Outlook and discussion

'-Omics' technologies provide a new opportunity to study complex diseases such as mental disorders through an integrated approach based on systems biology. These techniques may extend our view beyond present hypotheses. Non-hypothesis driven research is especially needed to solve those clinical questions where hypothesis-driven research has failed up to now. Although biomarkers for diagnostic and treatment purposes should preferably have an etiology-supported basis, their discovery might be through the use of techniques without any etiological assumption on forehand, at least theoretically. The needs of the

psychiatric community in biomarkers that aid in providing directions to the most (cost-) effective treatment with the least side-effects drive this research. A treatment biomarker may even turn out to be responsive to psychosocial therapy. In the end it may be possible to predict the onset of a disorder, to relate alterations in a causative manner to symptoms, and to define markers for recovery, remission and therapeutic response. Of course, all this is not possible without an extensive network that supports biomarker discovery, validation and implementation. Especially in mental disorders of low prevalence, international collaboration is required. The mental disorder biomarker discovery network has to extend from policy-makers that enable the collection of human material in large population cohorts and that provide financial support to researchers, to the end-users that benefit from the outcomes (i.e. the affected individual and his/her family). Technological advances in the field of genomics, transcriptomics, proteomics and metabolomics have been significant and it seems that there are mainly logistic and financial limitations to exploit these techniques in psychiatry research in a manner that is presently done in cancer. Results from cancer research are promising, but a marker discovered by '-omics' approaches has yet to conquer a place in the diagnostic arsenal of a clinical chemistry laboratory.

6. Conclusion

Mental disorders cause enormous psychosocial and socioeconomic suffering to those that are affected and to those living close by (e.g. relatives, friends and neighbors). Future projections about the incidence and prevalence of mental disorders are pessimistic with e.g. predicted increases in unipolar depression. A major problem of most mental disorders is their chronic nature, resulting in impairment of normal living (e.g. in work, relations, family-life etc.) after onset until death. The chronic nature of mental disorders is probably the most important contributor to the large burden-of-disease. For some disorders such as schizophrenia, abnormal behavior may already be present before the disorder is recognized and treated. For autism, children are impaired already from the early start of their life. Significant underdiagnosis and undertreatment in psychiatry is a fact of live, especially in third-world countries. This is mainly due to unequal distribution of psychiatric resources such as medication and the number of psychiatrists per capita. We still lack objective and quantifiable diagnostic measures, such as biological markers. Even in western countries available treatment modalities, either pharmacological or psychosocial, are far from being effective and most patients have residual symptoms for the rest of their lives or experience relapse. More insight into the causative mechanisms and risk factors may enable us to increase the efficacy and reduce the toxicity (e.g. metabolic side effects of

antipsychotics) of existing drugs, or to generate novel pharmacological targets. It is important to account for early-life events and environmental factors as wells as for genetic and epigenetic influences equally when investigating mental disorders. Especially adverse early-life events seem to do considerable harm, thereby significantly increasing the risk of developing a psychiatric disorder. Defining risk factors at different stages of life, ranging from optimal guidance of pregnancies to the prevention of depression in the elderly, through epidemiological research seems fruitful. If it is possible to link these risk factors in a mechanistic way to pathology, we may be able to intervene on a population level or set-up screening programs that track-down high-risk individuals. One possible way by which we can relate risk-factors to mechanistic pathways that cause disease is through the use of well-established '-omics' technologies (genomics, transcriptomics, proteomics and metabolomics). These unbiased (non-hypothesis driven) profiling techniques promise to extend our knowledge through systems biology-based mental disorder research. However, reflection upon the results of proteomics studies in cancer show that the field of biomarker discovery and validation is still in its infancy with regard to the number of biomarkers that have reached the clinic. It seems that, metaphorically speaking, the ripe fruits of knowledge and biomarkers have yet to be harvested from the tree of '-omics' technologies, and these fruits need to be undone of bugs and leaves before they can be eaten. Nevertheless, it is expected that if we combine the results from 'classic' hypothesis-driven experiments with those from unbiased non-hypothesis driven profiling techniques, it will be possible to improve the prospects of people suffering from these devastating disorders.

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Glossary (see also 'List of abbreviations')

abbreviation		*explanation*
ASD	autism spectrum disorders	see 'PDD'
DALY	disability-adjusted life year	a time-based measure that combines, in a single indicator, years of life lost from premature death and years of life lived with a disability
LMR	lifetime morbid risk	theoretical prevalence at any point in life for anyone, regardless of time of assessment
NPV	negative predictive value	proportion of patients with negative test results who are correctly diagnosed
PDD	pervasive developmental disorders	umbrella-term encompassing autistic disorder, Asperger's disorder, Rett's syndrome, childhood disintegrative disorder and PDD-not otherwise specified
PPV	positive predictive value	proportion of patients with positive test results who are correctly diagnosed
	endophenotype	heritable, measurable traits or facets in between genotype and phenotype reflecting neurobiological features underlying a readily apparent disease
	evolutionary medicine	study of present medical conditions in the context of possible discrepancies between current human environments and behaviors and past conditions under which we evolved
	sensitivity	true positive rate: proportion of true positives of all positive (e.g. diseased) cases in the population/sample
	specificity	true negative rate: proportion of true negatives of all negative (e.g. healthy) cases in the population/sample